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THE EFFECT OF LEAF RUST ACCOMPANIED BY HEAT UPON YIELD, KERNEL WEIGHT, BUSHEL WEIGHT, AND PROTEIN CONTENT OF HARD RED SPRING WHEAT[1]

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INTRODUCTION

This paper presents evidence from 4 families from the Ceres × (Hope × Florence) cross comprising a total of 80 selections. These were grown during 2 successive years in experiments to determine yield and other characters. A comparison of these characters for the 2 years shows a marked difference among the families in respect to yield, weight per 1,000 kernels, weight per bushel, and protein content. The weather for the 2 years was similar except that, principally, the July heat in 1935 was decidedly above that in 1934. As the four families differed from one another in resistance to leaf rust and in resistance to flecking, but did not differ greatly in these characters from one season to the other, it is evident that a measure exists by which to determine the injury brought about by flecking and by leaf-rust lesions under conditions of heat well above the optimum for the development of the wheat plant. The results of such measurements are given.

PREVIOUS RESULTS

During the past few years valuable results have been secured by various workers regarding the effect of leaf rust (*Puccinia triticina* Eriks.) upon cereals, particularly with respect to wheat (*Triticum aestivum* L.). It has been recognized from observation that leaf rust upon wheat reduces the yield, and controlled experiments both in the field and greenhouse, such as those conducted by Caldwell et al. (*1*)[2], Mains (*5*), Johnston and Miller (*3*), and others, have given definite information upon this point. Reduction of yield comes about in different ways, but if the plant becomes infected in the earlier stages of its life injury is evident mainly in the formation of fewer and smaller kernels. If the leaf rust attack comes mainly after the blossoming stage then the damage is largely confined to reduced kernel size with a consequent effect upon yield. The effect of leaf rust upon yield per acre and weight of kernel as determined by various individuals is shown in table 1.

Reductions in yield were analyzed by Johnston and Miller, for example, and the yield loss was found to be associated with a decrease in both number and size of kernels. The experimental plants were inoculated at different stages and the greatest losses in yield were found in those groups receiving the earlier inoculations, as might have

[1] Received for publication Feb. 24, 1936; issued October, 1936. Paper no. 19 of the Journal Series of the North Dakota Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 413.

TABLE 1.—*Effect of leaf rust on weight of kernel and yield as secured by various investigators*

Author	Variety	Type	Location	Rust in plant groups		Kernel weight				Loss (− or gain (+) in yield
				Control	Rusted	Control	Rusted	Loss in weight		
				Percent	*Percent*	*Milligram*	*Milligram*	*Milligram*	*Percent*	*Percent*
Johnston (*2*)	Malakof	Susceptible	Greenhouse	0	65	30.0	26.0	4.0	13.3	−55.7
	Fulhard	Resistant	do	0	[1]	30.0	29.0	1.0	3.3	−22.3
	Prelude	Susceptible	Field [2]	26	72	21.5	20.9	.6	2.8	
	Turkey	do	do	20	65	21.1	17.4	3.7	17.5	
Mains (*5*)	Red Fern	do	Greenhouse	0–35	15–85	28.3	21.9	6.4	22.6	−57.2
	Mediterranean	do	do	0–35	10–85	22.9	16.7	6.2	27.1	−63.3
	Fulcaster	do	do	0–5	75–100	35.9	28.9	7.0	19.5	−85.2
	Michigan Amber	do	do	0–1	100	35.8	29.8	6.0	16.8	−33.5
	do	do	do	0–1	75–100	33.0	23.7	9.3	28.2	−37.2
	do	do	do	0–T	90–100	36.6	29.6	7.0	19.1	−27.2
Johnston and Miller (*3*)	Illinois No. 1	do	Field	T–35	75–100	30.0	27.5	2.5	8.3	−24.2
	Webster	Resistant	Greenhouse	0	[1]	39.6	32.3	7.3	18.4	−11.4
	Pusa	Susceptible	do	0	85	45.0	32.6	12.4	27.6	−75.4
	Warden	Resistant	do	0	17	37.2	33.9	3.3	8.9	−7.0
Caldwell et al. (*1*)	Shepherd	Susceptible	Field	13	100	33.4	30.9	2.5	7.5	−28.4
	Gladden	do	do	15	100	35.6	31.3	4.3	12.1	−14.8
	Fulhard [3]	do	do	10	100	32.6	30.9	1.7	5.2	+1.2
	Nittany	do	do	10	75	42.7	41.4	1.3	3.0	−8.2
	Purkof	do	do	8	65	32.8	31.2	1.6	4.9	−2.0
	Kawvale	Semiresistant	do	5	45	33.9	32.8	1.1	3.2	+3.4
	Fultz selection	Resistant	do	1	7	32.4	32.0	.4	1.5	+12.9
Weiss (*10*)	Marquis	Susceptible	Greenhouse	.5	10					−10.1

[1] Flecked.
[2] Control plants in the field were sulphur-dusted; those in the greenhouse were uninoculated.
[3] Very badly rusted, not resistant.

been expected. A part of the yield reduction was due to decreased weight of kernel, but this reduction in kernel weight did not come about by shriveling, as mainly happens with stem rust (*Puccinia graminis tritici* Eriks. and Henn.), but plumpness was largely retained at the expense of size. This is clearly brought out by the different investigators.

In the experiments cited no mention is made of the relation of temperature to the effects of leaf rust, as this factor did not enter into the experiments summarized in table 1. It is well known that temperature exerts an important influence upon the development of rust, both leaf and stem. Within limits, increasingly high temperatures lessen the time of incubation and result in other modifications, with the usual tendency to increase the virulency of an epidemic. While Lambert (*4*), Peltier (*7*), Melander (*6*), and others have presented observations upon the effect of temperature on the seriousness of stem rust epidemics, but little experimental work appears to have been carried out under controlled conditions of temperature to show the net effect of rust upon the crop in the field under temperatures appreciably divergent. The relation of the rust parasite to its host is complex and the end result is affected by various environmental and internal factors. These have been studied more or less apart from each other, but the integrated effect of such factors upon the host can be determined finally only by empirical methods, particularly under field conditions.

Results secured by the writer with some Ceres × (Hope × Florence) hybrids during the years 1934 and 1935 are of interest in this connection because controlled conditions were rather closely simulated. The hybrids which are of particular interest comprise four families which show different reactions to leaf rust. The reactions to stem rust also differ among the families, but these appear to be of minor importance in this experiment. One of the families, no. 60.4, shows high resistance to leaf rust and even markedly resists flecking. Family no. 54.2 has shown marked resistance to leaf rust at Fargo, North Dakota, but is less resistant to flecking than family 60.4. The two other families, nos. 81.1 and 81.22, are moderately susceptible to leaf rust.

WEATHER IN 1934 AND 1935

The growing seasons of 1934 and 1935 at Fargo were both dryer and hotter than normal growing seasons. In 1934 the excessive heat was in May and July, while in 1935 the high temperatures were limited to July but the heat of that month was excessive. A severe epidemic of stem rust did great damage to the wheat crop in 1935, but evidently it had only a minor effect upon the wheats under discussion as they all showed high resistance. The normal daily mean temperatures for Moorhead, Minn., about 2 miles from the experiment, are given in table 2 in averages of 5-day periods, pentads, and these values are compared with similar average daily means for 1934 and 1935 for the months of May to July. Five-day precipitation totals are also given.

TABLE 2.—*5-day (or 6-day) average daily mean temperatures from May to July 1934 and 1935 compared with similar normal daily mean temperatures and similar data dealing with 5-day precipitation totals* [1]

Period	Temperature					Precipitation		
	Normal	1934	1935	Deviation from normal				
				1934	1935	Normal	1934	1935
	°F.	°F.	°F.	°F.	°F.	*Inches*	*Inches*	*Inches*
May 1–5	49.7	57.8	40.2	8.1	−9.5	0.39	0.79	0.66
May 6–10	51.5	67.8	48.8	16.3	−2.7	.42	.08	.36
May 11–15	54.0	52.2	46.4	−1.8	−7.6	.40	.02	.62
May 16–20	55.9	71.0	58.4	15.1	2.5	.51	.14	0
May 21–25	57.9	53.2	57.6	−4.7	−.3	.46	0	.14
May 26–31	60.0	77.2	57.5	17.2	−2.5	.57	.12	.03
Total						2.75	1.15	1.81
June 1–5	61.7	68.4	52.6	6.7	−9.1	.51	.73	.36
June 6–10	62.5	59.4	58.0	−3.1	−4.5	.60	1.42	.17
June 11–15	63.9	60.8	66.4	−3.1	2.5	.68	0	.95
June 16–20	65.8	66.4	61.8	.6	−4.0	.62	1.05	0
June 21–25	66.1	69.6	64.4	3.5	−1.7	.64	.70	1.53
June 26–30	67.3	72.0	68.4	4.7	1.1	.78	.03	0
Total						3.83	3.93	3.01
July 1–5	67.8	68.0	76.8	.2	9.0	.74	.28	.01
July 6–10	68.5	68.4	70.4	−.1	1.9	.48	.04	.83
July 11–15	68.9	72.2	72.4	3.3	3.5	.57	.36	.74
July 16–20	69.3	78.8	77.8	9.5	8.5	.59	0	.04
July 21–25	69.0	80.0	77.0	11.0	8.0	.38	.10	.12
July 26–31	68.5	68.7	78.0	.2	9.5	.53	.02	.39
Total						3.29	.80	2.13
Average or total	62.7	67.3	62.9	4.6	.3	9.87	5.88	6.95

[1] Data are from the official records of the U. S. Weather Bureau.

During May 1934 there were three 5-day periods of excessive heat with one other well above the average. Rains meanwhile were deficient. Both May and June, 1935, were definitely below normal in temperature, the daily deficiency averaging 3°. June temperatures for 1934 were 1.6° above normal. July in 1934 and again in 1935 had temperatures decidedly above normal; these averaged +4° daily for 1934 and +6.7° for 1935. The absolute maxima for July for the 2 years were 106° and 96° F., respectively. The 3 months in 1935 showed a decidedly lower daily average temperature than did the same months in 1934. The belief is common that in 1935 the small-seeded cereals, especially wheat, suffered more from excessive July heat than they did in 1934. There is evidence that this belief is founded in fact, as will appear later, yet the daily excess temperature for July 1935 was but 2.7° above the daily average for July 1934. Also, the crop in 1934 received decidedly more heat during its post-heading period than was the case in 1935. It has been suggested that the marked damage in 1935 may have been associated with the profound change from the very cool weather of May and June to the excessive heat of July, and the inability of the plants to adapt themselves to the radically new environment. While this conclusion is rather speculative it is well to give it consideration. The presence of the stem rust epidemic makes it difficult to evaluate the effect of the heat alone on most wheat varieties.

EXPERIMENTAL PROCEDURE

In 1934, 24 selections were taken of each of the 4 families mentioned above and 1 other family [3] and grouped into 6 experimental units each of which carried 4 hybrid selections from each family plus a check variety grown four times or 2 checks each grown 2 times. Each experimental unit thus contained 24 wheats from which yields were to be obtained. In each experimental unit each selection or check was triplicated and the rod rows were arranged in a random manner. In 1935 one of the six experimental units was located some distance from the other five on a different soil type. Because the yield of this unit was decidedly below that of the rest of the experiment it was omitted, as was the corresponding experiment of 1934. This left 5 experimental units for each year, each unit of which contained 20 of the hybrids plus 4 checks. In this paper, for purposes of comparison, the one family indicated and the checks are omitted. Thus there are 80 of the Ceres × (Hope × Florence) hybrid selections for comparison grouped into 4 families grown in duplicate during tne 2 years, plus the 4 checks for each experimental unit. There was a slight change in the checks from the one year to the next, and in 1935, contrary to the practice in 1934, the yield rows were guarded. A study of the statistical constants of the two experiments shows that the absence of the guard rows in 1934 does not invalidate the comparisons which are made here.

EXPERIMENTAL RESULTS

YIELD AND WEIGHT DATA

The detailed data with respect to yield, weight per 1,000 kernels, and weight per bushel of the selections of the four families for the 2 years, and the respective leaf rust readings are shown in table 3.

The data for each family of selections are arranged together even though different experimental units are concerned, as all units were given comparable treatment. While yields are distinctly lower for 1935 for three of the families they are essentially the same for family 60.4. In this family for 1935 a trace of leaf rust is reported in each case, but these readings are probably too conservative and a zero reading would have been more nearly correct. Plants showing even minute pustules were unusual. No more than a trace of stem rust was observed in 1934.

The results can be better appreciated when summarized as in table 4.

[3] This other family, no. 81.45, is omitted as it is believed that its yields in 1934 were relatively low because of a thin stand, which may have resulted in part from thin seeding due to very large kernels. It is not evident the omission of this family prejudices the study in any way.

TABLE 3.—*Data on 4 families of Ceres × (Hope × Florence wheat) with respect to leaf and stem rust, yield, kernel weight, and weight per bushel for 1934 and 1935*

Experiment no.	Family and selection no.	Leaf rust		Stem rust, 1935	Yield per acre			Weight per 1,000 kernels			Weight per bushel		
		1934	1935		1934	1935	Difference	1934	1935	Difference	1934	1935	Difference
	Family 54.2:	*Pct.*	*Pct.*	*Pct.*	*Bu.*	*Bu.*	*Bu.*	*G.*	*G.*	*G.*	*Lb.*	*Lb.*	*Lb.*
1	1	1	1	2	38.3	35.2	−3.1	33.1	27.1	−6.0	59.0	56.0	−3.0
2	3	1	3	1	35.7	28.3	−7.4	33.1	26.6	−6.5	59.5	54.5	−5.0
3	5	1	1	2	37.5	32.8	−4.7	32.4	27.5	−4.9	57.5	56.0	−1.5
4	6	1	1	3	38.8	27.7	−11.1	33.3	27.2	−6.1	59.5	55.0	−4.5
5	7	T	1	2	40.5	24.6	−15.9	30.2	24.7	−5.5	59.0	55.0	−4.0
6	8	T	1	4	41.9	30.1	−11.8	34.0	27.3	−6.7	59.0	55.0	−4.0
7	9	T	2	3	39.4	30.4	−9.0	32.4	29.0	−3.4	58.5	54.0	−4.5
8	10	T	1	3	37.5	27.7	−9.8	31.2	27.7	−3.5	58.5	55.5	−3.0
9	11	T	4	3	35.7	35.0	−.7	31.6	28.3	−3.3	58.0	55.5	−2.5
10	14	T	1	2	37.6	30.4	−7.2	30.3	27.0	−3.3	58.5	55.5	−3.0
11	15	T	1	2	35.3	32.3	−3.0	31.7	26.6	−5.1	58.0	54.5	−3.5
12	16	1	1	1	38.9	29.1	−9.8	30.7	26.3	−4.4	58.0	55.0	−3.0
13	17	T	1	2	36.1	32.6	−3.5	31.4	27.2	−4.2	58.0	55.0	−3.0
14	18	T	T	2	36.9	31.9	−5.0	31.3	24.4	−6.9	58.0	56.0	−2.0
15	19	T	1	2	37.1	31.8	−5.3	30.5	26.6	−3.9	58.5	55.5	−3.0
16	21	T	T	3	37.7	34.1	−3.6	32.3	28.2	−4.1	58.5	56.0	−2.5
17	22	3	5	1	29.8	31.6	+1.8	31.9	24.7	−7.2	58.0	55.0	−3.0
18	23	1	1	2	34.4	31.8	−2.6	32.9	25.8	−7.1	58.5	55.0	−3.5
19	24	1	2	1	34.7	29.6	−5.1	28.9	23.5	−5.4	57.5	53.5	−4.0
20	25	2	2	1	34.2	32.5	−1.7	32.4	26.5	−5.9	58.0	54.5	−3.5
	Average	0.6	1.5	2.2	36.9	31.0	−5.9	31.8	26.6	−5.2	58.4	55.1	−3.3
	Family 60.4:												
1	1	T	T	0	35.4	32.8	−2.6	34.5	31.6	−2.9	59.0	56.5	−2.5
2	2	T	T	1	38.3	33.2	−5.1	35.1	30.8	−4.3	59.5	56.0	−3.5
3	3	T	T	T	33.5	39.5	+6.0	35.2	30.8	−4.4	60.0	58.5	−1.5
4	4	T	T	2	35.6	35.7	+.1	34.5	30.7	−3.8	59.5	56.0	−3.5
5	5	T	T	2	41.5	32.0	−9.5	33.0	26.3	−6.7	59.5	54.5	−5.0
6	6	1	T	3	32.3	34.3	+2.0	34.1	30.2	−3.9	58.5	56.5	−2.0
7	7	T	T	2	35.2	37.2	+2.0	34.7	32.6	−2.1	59.0	56.5	−2.5
8	8	T	T	5	43.8	34.0	−9.8	34.5	29.3	−5.2	59.0	55.5	−3.5
9	9	T	T	2	36.5	34.5	−2.0	33.1	31.1	−2.0	59.0	57.0	−2.0
10	10	T	T	T	32.0	33.2	+1.2	31.5	27.4	−4.1	58.5	56.5	−2.0
11	11	T	T	T	35.1	35.6	+.5	32.5	31.4	−1.1	58.5	58.0	−.5
12	12	T	T	T	35.7	34.5	−1.2	30.7	32.3	+1.6	59.0	57.5	−1.5
13	13	T	T	2	32.8	32.8	0	32.2	31.0	−1.2	59.0	58.0	−1.0
14	14	T	T	1	32.8	35.8	+3.0	31.5	29.4	−2.1	58.5	56.5	−2.0
15	15	T	T	3	34.3	36.5	+2.2	32.8	29.8	−3.0	59.0	58.0	−1.0
16	16	T	T	2	36.6	37.1	+.5	31.1	31.6	+.5	59.0	57.5	−1.5
17	17	T	T	3	28.7	36.5	+7.8	32.8	33.0	+.2	57.5	57.5	0
18	18	1	T	1	27.8	36.1	+8.3	32.0	28.9	−3.1	58.0	56.5	−1.5
19	19	T	T	T	34.8	40.8	+6.0	32.8	32.3	−.5	57.5	56.5	−1.0
20	20	T	T	2	32.9	34.8	+1.9	31.5	26.7	−4.8	58.5	56.0	−2.5
	Average	T	T	1.6	34.8	35.5	+0.7	33.0	30.4	−2.6	58.8	56.8	−2.0
	Family 81:1:												
1	1	2	3	T	37.8	31.8	−6.0	38.3	29.6	−8.7	60.0	56.0	−4.0
2	2	2	4	T	38.4	30.1	−8.3	35.2	30.6	−4.6	60.0	57.5	−2.5
3	3	11	23	T	37.7	28.0	−9.7	37.6	24.6	−13.0	59.5	53.5	−6.0
4	5	7	10	1	37.7	29.2	−8.5	37.8	25.7	−12.1	60.0	54.0	−6.0
5	6	11	22	T	37.5	24.6	−12.9	37.1	25.7	−11.4	58.5	52.5	−6.0
6	7	11	18	T	37.3	26.1	−11.2	35.9	30.5	−5.4	59.5	54.0	−5.5
7	8	11	17	T	36.9	27.1	−9.8	33.8	22.3	−11.5	59.5	53.5	−6.0
8	9	8	13	T	34.6	27.9	−6.7	36.0	31.8	−4.2	59.5	54.0	−5.5
9	10	5	7	T	35.9	30.9	−5.0	35.2	28.1	−7.1	58.0	55.0	−3.0
10	11	3	10	T	36.1	31.8	−4.3	33.5	28.2	−5.3	59.0	55.5	−3.5
11	12	4	10	T	33.4	30.1	−3.3	34.2	26.3	−7.9	59.0	56.0	−3.0
12	13	2	11	T	34.5	30.3	−4.2	34.5	26.6	−7.9	58.5	55.0	−3.5
13	14	6	7	T	35.4	23.2	−12.2	33.4	22.5	−10.9	59.0	54.5	−4.5
14	15	5	6	T	36.4	27.2	−9.2	33.1	24.0	−9.1	59.0	55.0	−4.0
15	16	2	5	T	36.5	33.5	−3.0	35.0	28.6	−6.4	58.5	57.0	−1.5
16	17	7	22	T	33.0	25.4	−7.6	32.6	25.4	−7.2	58.5	55.0	−3.5
17	18	6	13	T	33.1	27.8	−5.3	32.9	26.2	−6.7	59.0	54.5	−4.5
18	19	4	16	T	33.0	29.8	−3.2	32.1	22.8	−9.3	59.0	55.0	−4.0
19	20	3	6	T	32.4	29.6	−2.8	33.8	25.6	−8.2	58.0	54.5	−3.5
20	21	4	23	T	30.6	26.6	−4.0	37.1	25.6	−11.5	58.5	53.5	−5.0
	Average	3.7	12.3	T	35.4	28.5	−6.9	35.0	26.5	−8.5	59.0	54.8	−4.2

TABLE 3.—*Data on 4 families of Ceres × (Hope × Florence wheat) with respect to leaf and stem rust, yield, kernel weight, and weight per bushel for 1934 and 1935*—Continued

Experiment no.	Family and selection no.	Leaf rust		Stem rust, 1935	Yield per acre			Weight per 1,000 kernels			Weight per bushel		
		1934	1935		1934	1935	Difference	1934	1935	Difference	1934	1935	Difference
	Family 81.22:	*Pct.*	*Pct.*	*Pct.*	*Bu.*	*Bu.*	*Bu.*	*G.*	*G.*	*G.*	*Lb.*	*Lb.*	*Lb.*
1	1	6	15	T	35.1	25.0	−10.1	35.5	26.2	−9.3	60.5	56.0	−4.5
2	2	11	13	T	37.8	25.0	−12.8	37.4	23.2	−14.2	61.0	56.5	−4.5
3	3	8	18	T	37.1	29.0	−8.1	33.6	24.5	−9.1	61.0	56.5	−4.5
4	4	9	15	T	38.2	28.0	−10.2	34.6	23.3	−11.3	61.0	54.5	−6.5
5	5	8	12	1	39.9	27.1	−12.8	33.9	25.4	−8.5	61.0	56.0	−5.0
6	7	13	13	3	40.5	26.6	−13.9	33.2	23.6	−9.6	61.0	56.0	−5.0
7	8	5	9	1	41.3	24.6	−16.7	34.3	22.8	−11.5	60.5	55.5	−5.0
8	9	6	9	T	41.7	30.0	−11.7	35.7	25.1	−10.6	60.5	56.0	−4.5
9	10	5	8	T	36.9	26.2	−10.7	33.1	24.2	−8.9	60.0	55.5	−4.5
10	12	4	12	T	37.3	28.4	−8.9	32.5	24.8	−7.7	60.0	56.5	−3.5
11	13	4	7	T	37.0	27.2	−9.8	31.5	23.3	−8.2	60.0	57.0	−3.0
12	14	5	11	T	35.2	21.5	−13.7	33.2	23.3	−9.9	59.5	53.5	−6.0
13	15	5	15	T	37.1	27.6	−9.5	32.4	23.7	−8.7	60.0	55.0	−5.0
14	16	8	18	T	36.7	26.7	−10.0	33.3	25.0	−8.3	59.5	54.5	−5.0
15	17	5	12	T	37.0	27.5	−9.5	32.6	24.0	−8.6	60.5	56.5	−4.0
16	19	8	15	1	40.1	27.1	−13.0	32.9	23.9	−9.0	59.5	54.5	−5.0
17	20	7	15	T	36.8	27.1	−9.7	32.2	22.2	−10.0	60.0	53.5	−6.5
18	21	7	18	T	33.0	29.5	−3.5	31.7	22.1	−9.6	60.0	55.0	−5.0
19	22	6	15	T	33.5	28.8	−4.7	31.9	22.1	−9.8	60.0	55.5	−4.5
20	23	10	15	T	35.0	25.3	−9.7	30.6	21.7	−8.9	60.0	54.0	−6.0
	Average	7.0	13.2	0.3	37.4	26.9	−10.5	33.3	23.7	−9.6	60.3	55.4	[2]−4.9

TABLE 4.—*Quantitative changes summarized from table 3 in some Ceres × (Hope × Florence) hybrid families from 1934 to 1935*

YIELD PER ACRE

Family	1934				1935					Increase or decrease in 1935	
	Leaf rust [1]	Mean	Standard error		Rust		Mean	Standard error			
					Leaf	Stem					
	Percent	*Bushels*	*Value* [2]	*Percent* [3]	*Percent*	*Percent*	*Bushels*	*Value* [2]	*Percent* [3]	*Bushels*	*Percent*
60.4	Trace	34.8	0.83	10.36	Trace	1.6	35.5	0.50	6.16	0.7	2
54.2	0.6	36.9	.59	6.92	1.5	2.0	31.0	.59	8.31	−5.9	−16
81.1	5.7	35.4	.49	6.09	12.3	Trace	28.5	.59	8.99	−6.9	−19
81.22	7.0	37.4	.54	6.26	13.2	.3	26.9	.44	7.14	−10.5	−28
WEIGHT PER 1,000 KERNELS											
		Grams					*Grams*				
60.4	----------	33.0	0.32	4.16	----------	----------	30.4	0.42	6.08	−2.6	−8
54.2	----------	31.8	.28	3.84	----------	----------	26.6	.31	5.13	−5.2	−16
81.1	----------	35.0	.42	5.23	----------	----------	26.5	.61	10.05	−8.5	−24
81.22	----------	33.3	.36	4.70	----------	----------	23.7	.27	4.98	−9.6	−29
WEIGHT PER BUSHEL											
		Pounds					*Pounds*				
60.4	----------	58.8	0.14	1.06	----------	----------	56.8	0.22	1.66	−2.0	−3
54.2	----------	58.4	.13	.96	----------	----------	55.1	.15	1.20	−3.3	−6
81.1	----------	59.0	.14	1.02	----------	----------	54.8	.27	2.17	−4.2	−7
81.22	----------	60.3	.12	.85	----------	----------	55.4	.23	1.84	−4.9	−8

[1] Stem rust essentially zero in 1934. [2] Standard error for family mean. [3] Equals variability.

LEAF RUST READINGS

An attempt was made to read a pustuled area with a reasonable degree of accuracy. A leaf, dried, was taken from one of the selections of family 81.22 and a portion measuring 11 by 20 mm was marked off into 20 squares and viewed with a low-power compound microscope. The total area was sampled, once for each square, and the area of each pustule in each field measured approximately by a micrometer scale. The 20 fields covered 76.03 mm^2 of leaf surface, of which 5.55 mm^2, or 7.3 percent, was found to be pustular. As these pustules had passed into the teliospore stage their outlines were fairly definite but in their subepidermal condition the edges were indistinct. According to the standard adopted by the Division of Cereal Crops and Diseases, United States Department of Agriculture, and commonly used in rust readings, 7.3 percent of the area covered would be equal to a rust reading of 20 percent. This particular selection had a leaf-rust reading estimated in the field as 13 percent. Accurate reading upon a single leaf blade might easily show much divergence from an estimated average for the selection. However, the pustules observed under the microscope were small. Nearly one-third of the estimated total of 446 found on the 220 mm^2 had an average area of but 0.013 mm^2, equal to a circle diameter of 0.13 mm. Pustules of such small size would escape attention in ordinary rust readings. A microscopic examination of leaves from family 60.4 failed to show any pustules, although certain of the relatively few minute yellowed areas were probably flecks. Pustules on leaves of family 54.2 could be found, although they were far less common and of smaller size than on leaves of family 81.22. Flecking on these leaves was markedly greater than on leaves of family 60.4 but less than on those of family 81.22.

COMPARISON OF 1934 AND 1935 RESULTS

It is evident that the work described above was not carried out under controlled conditions in the usual sense of the term. One variety is not subject to the presence and absence of leaf-rust infection under two controlled conditions of temperature. In lieu of this the two temperature gradients are supplied by growing identical crops under similar conditions for 2 successive years which differ materially, and probably mainly, in the amount and rate of heat delivered to the crop during the time required for the disease to develop. In place of one variety divided in such a manner that a portion becomes rusted, we have here closely related families which differ in their reactions to rust.

The results for 1934 show that the four families behaved similarly as to yield and other indicated characters. Any disparities in 1934 may be explained in part by accidental conditions, such as a slight shattering in family 60.4. The same wheats showed a very different behavior in 1935. The question then arises: Is this difference due to the combined effect of leaf rust and heat in 1935 or would the disparities of 1935 have appeared had there been the increased heat without the leaf rust? While opinion may differ, the evidence indicates that leaf rust, associated with high temperatures, was the responsible agent. In short, the results presented herewith may be considered essentially equivalent to those of a controlled experiment.

The crop was subjected to the entire weather picture, and obviously there are fewer gaps to bridge when conclusions are extended to field results than if the extension were to be made from greenhouse experiments.

Stem rust was low in 1935 for all families, the maximum average reading being 2.2 percent for family 54.2. Injury resulting from an amount of rust so low as this is rather minor. Most of the rust was located near the nodes and thus translocation was not much disturbed. The families are placed in ascending order of leaf-rust reading and the 1935 yields fall into a contrary order. The yield of family 60.4 was significantly below that of family 81.22 in 1934. This was partly due to the fact that there was a little shattering of 60.4 in 1934. Moreover, this family was cut somewhat earlier in 1934 because of its tendency to shatter. Judging from the decrease of 8 percent in weight per 1,000 kernels and of 3 percent in weight per bushel of family 60.4 from 1934 to 1935, the yield of this family probably suffered in 1935 because of high temperatures. The 1934 yield also probably suffered because of the reduction mentioned from the optimum otherwise obtainable.

The difference in leaf rust in 1935 of 1.5 percent between families 60.4 and 54.2 is apparently too small to influence yield as a direct result of the parasite. As the weight per 1,000 kernels and weight per bushel are both lower for family 54.2, the major portion of the difference in yield of 4.5 bushels is pretty certainly due to seasonal effect. In 1934 family 60.4 yielded 2.1 bushels less than family 54.2. The major factor which brought about a relative reduction of yield in family 54.2 as compared to family 60.4, in 1935, is believed to have been the deterioration of the leaf due to the flecking caused by leaf rust. This premature yellowing of the leaf before the normal ripening of the plant, which was probably followed by necrosis and which interfered with normal photosynthesis, naturally affected seed production. With the greater amount of leaf rust in the other two families, 81.1 and 81.22, the deterioration of the leaf was carried still farther and the effect upon the crop was still greater. In the present instance it does not seem possible to distinguish between the injury brought about incidentally by the parasite as a result of flecking, commonly followed by necrosis, and the direct effect of the parasite, in the utilization of food of the host. A study of the mature plants of families 81.1 and 81.22 leads to the belief that the latter family was subject to greater infection in 1935, although table 3 shows that infection upon the leaf blade, used in making the readings, was nearly the same in the two cases. Family 81.22 appeared to carry more leaf-rust pustules upon the sheaths and upon the peduncles, but it is not easy to obtain quantitative evidence on this point. If a real difference existed in this respect we would probably find here an explanation of the much smaller yield of family 81.22 as compared with family 81.1. One cannot be certain that the pathogen continued to act injuriously upon the host until the latter was nearly or quite mature but this seems a reasonable supposition.

The yields and weights per 1,000 kernels for 1935 reduced to percentages with family 60.4 as the base are shown in table 5.

Families 54.2 and 81.22 show almost exact proportional deficiency in yield and weight of kernel when compared with family 60.4. Evi-

dently differences in yield are attributable mainly to those factors which brought about a smaller kernel. The difference between families 81.1 and 60.4, in respect to yield is perhaps significantly greater than in respect to kernel weight.

The weights per bushel show much smaller differences among each other than the corresponding ones for yield and kernel weight. Family 60.4 has the highest bushel weight, 56.8 pounds, which is 2 pounds, or 3 percent, lower than its bushel weight for 1934. This is probably a fair indication of the loss that resulted from the excess heat of 1935. The lower yielding families of 1935 had bushel weights lower than that of family 60.4. While the maximum differences with family 81.1, was but 2 pounds, or 3.5 percent, statistically this is strikingly significant. The odds are several million to one against the probability that a mean deviation as large as 2 pounds could accidentally arise.

TABLE 5.—*Yields and weights per 1,000 kernels reduced to percentages with the results for family 60.4 as a base, 1935*

Family	Yield	Weight of 1,000 kernels	Family	Yield	Weight of 1,000 kernels
	Percent	*Percent*		*Percent*	*Percent*
60.4	100	100	81.1	80	87
54.2	88	87	81.22	76	78

Few results are found in the literature concerning the effect of leaf rust on bushel weight. Caldwell et al. (*1*) secured bushel weights on winter wheat grown outside which had been sulphur-dusted to control leaf rust. Omitting two varieties which showed reversal of yields, perhaps due to sulphur injury, they found that the weight of the grain produced by the five varieties on the control plots was 59.9 pounds per bushel, while the bushel weight from the corresponding rusted plots was 59.0 pounds. The corresponding loss in yield of grain averaged 11 percent. The leaf-rust readings averaged 90 and 11 percent, respectively, for the rusted and control plots. While the rust readings in the experiments cited were much higher than in the present work, the falling off in yields from family 60.4 to the average of the three other families is 18 percent as compared to 11 percent in the winter wheat experiment. The change in bushel weight of 1.7 pounds, or 3 percent, from family 60.4 to the average of the three other families, is perhaps comparable to that secured by Caldwell and his associates.

A measure of injury resulting from a small amount of stem rust is obtainable from the present data. Family 60.4 contains 14 selections which averaged 2 percent of stem rust in 1935 while the other 6 carried no more than a trace. For convenience of study this family is divided into two groups, and the values are shown in table 6.

The group carrying 2 percent of stem rust in 1935 yielded 0.5 bushel more in 1934 and 1.1 bushels less in 1935 than the group carrying a trace of stem rust, a total difference of 1.6 bushels. Similarly, the kernel weight was 0.2 g higher in 1934 and 0.9 g lower in 1935, making a total difference of 1.1 g. The bushel weights were equal in 1934, but in the 1935 group with 2 percent rust weighed 0.7 pound less per bushel. These differences are small, but as they in-

TABLE 6.—*Separation of family 60.4 into two groups characterized by a trace and by 2 percent of stem rust in 1935*

Selection nos.	Rust			Yield per acre				Weight per 1,000 kernels				Weight per bushel			
	Leaf		Stem	1934	1935	Difference		1934	1935	Difference		1934	1935	Difference	
	1934	1935	1935												
	Pct.	*Pct.*	*Pct.*	*Bu.*	*Bu.*	*Bu.*	*Pct.*	*Grams*	*Grams*	*Grams*	*Pct.*	*Lbs.*	*Lbs.*	*Lbs.*	*Pct.*
9.60.4.1,3, 10–12, 19.	T	T	T	34.4	36.1	+1.7	+5	32.9	31.0	−1.9	−6	58.8	57.3	−1.5	−3
9.60.4.2, 4–9, 13–18, 20	T	T	2	34.9	35.0	+0.1	0	33.1	30.1	−3.0	−9	58.8	56.6	−2.2	−4

volve three characters they probably reflect the action of the parasite. No doubt under ordinary circumstances the effect of so little stem rust could not be detected in the plant, but with the high temperatures accompanying the 1935 epidemic this evidently became possible.

EFFECT OF HEAVY STEM RUST WITH LOW TEMPERATURES

It is a commonplace that if stem rust injures the wheat crop evidence of such injury appears in reduced yields and in shrunken kernels leading to reduced weights per bushel. However, a high bushel weight may accompany a rather high rust reading. In 1927 data were secured from the wheat nursery at Fargo which illustrate this point and are of particular interest here as they serve to throw light on the effect of rust on yield, and on bushel weight in this case, when accompanied by differences in temperature. The data are shown in table 7.

TABLE 7.—*Effect of leaf and stem rust on yield and bushel weight of various wheat varieties under cool temperature conditions, 1927*

Variety	Date of harvest	Rust		Yield per acre	Bushel weight
		Leaf	Stem		
		Percent	*Percent*	*Bushels*	*Pounds*
Marquis	Aug. 13	2	53	34.7	61.5
Ceres	Aug. 10	4	3	33.3	60.4
Axminster	Aug. 12	3	30	32.5	61.5
Reliance	Aug. 13	5	68	30.1	59.5
Renfrew	Aug. 14	2	75	22.7	53.0

Except for Ceres the stem rust readings on these wheats were high. Bushel weight and yield for Marquis and Axminster were not appreciably lowered as compared with those for Ceres. Evidently Reliance was somewhat adversely affected, but only Renfrew, a late and very susceptible variety, was seriously injured. In 1935 Marquis and Canus (the latter equivalent to Reliance) were grown in the nursery, and with rust readings at 65 and 75 percent, respectively, the respective yields were 7.7 and 6.8 bushels per acre and the respective bushel weights were 38.5 and 36.0 pounds.

The season of 1927 was cool, which caused the harvest to be nearly 2 weeks later than that of 1935. The average daily mean temperature from July 1 to August 13 was 2.8° F. below the normal daily mean, and only during the last 3 days of this period did the daily mean rise appreciably above the normal. This period of heat probably came too late to cause any marked rust injury. In 1935 the average daily mean temperature for July was 6.8° F. above the normal daily mean. Thus during the post-heading period for the two crops the difference of the two average daily means showed a daily disparity of nearly 10° F. Evidently, temperature exerts a very pronounced influence upon the severity of stem rust. An increase of heat rapidly increases the injury caused by the parasite. The same is evidently true of leaf rust. Injury from leaf rust may result directly from the action of the parasite or, indirectly, from the flecking produced by spore invasion which brings about a loss of chlorophyll tissue and often leads to extensive leaf yellowing and necrosis. In either case added heat results in increased loss.

RELATION OF LEAF RUST TO PROTEIN CONTENT

Caldwell et al. (*1*), in their studies of the effect of leaf rust upon the composition of winter wheat, found that the protein content was much affected, particularly so far as susceptible varieties were concerned. With increasing intensity of leaf rust the protein content in the grain decreased. In one instance the protein percentages were 11.5, 11.0, 10.5, and 10.2, as leaf-rust infection increased, either in amount of infection or in length of time infected. In the present study protein determinations [4] were made for 1934 and 1935, and the values secured are shown in table 8.

The first four protein determinations of family 54.2 for 1934 are decidedly below the other values in the column. The same is true for the corresponding top two values of family 60.4, the top four of family 81.1, and the top three of 81.22. These values are for wheats grown in a single experiment, and the reason for their unusual behavior with regard to protein is not clear. So far as known growth conditions were comparable for all of the selections grown in 1934. Comparisons were made with the values as they appear in the table and also when the 13 values indicated, with the corresponding 1935 values, are omitted. A study of the differences in protein content for the 2 years should show the effects of different amounts of leaf rust under different temperature conditions upon this character. The results are summarized in table 9.

[4] Acknowledgment is due Dr. C. E. Mangels, cereal chemist at the North Dakota station, for the protein analyses presented in this paper.

TABLE 8.—*Protein content, with differences, of four families of Ceres × (Hope × Florence) wheats grown comparably in 1934 and in 1935*

Family and selection no.	1934	1935	Difference	Family and selection no.	1934	1935	Difference
Family 54.2:				Family 81.1:			
1	13.8	16.4	2.6	1	13.5	16.0	2.5
3	14.1	16.7	2.6	2	14.4	15.9	1.5
5	13.7	16.2	2.5	3	13.8	1.2	1.4
6	13.7	15.9	2.2	5	14.0	16.2	2.2
9	15.1	16.5	1.4	6	15.7	15.1	−.6
10	15.2	16.0	.8	7	16.5	15.6	−.9
11	15.8	16.1	.3	8	15.9	15.2	−.7
14	15.7	16.1	.4	9	15.8	15.7	−.1
15	15.7	16.2	.5	10	16.3	15.5	−.8
17	16.0	16.5	.5	11	16.2	15.0	−1.2
18	15.7	16.0	.3	12	16.5	15.5	−1.0
19	15.9	16.5	.6	13	16.3	15.6	−.7
21	15.4	15.9	.5	14	16.0	15.0	−1.0
22	16.5	15.6	−.9	15	16.4	15.1	−1.3
23	15.9	15.8	−.1	16	16.2	15.3	−.9
24	15.7	16.2	.5	17	16.4	15.1	−1.3
25	15.9	16.0	.1	18	16.7	15.7	−1.0
				19	16.5	15.8	−.7
Average	15.3	16.2	.87	20	16.5	16.0	−.5
				Average	15.8	15.5	−.27
Family 60.4:							
2	13.7	15.5	1.8	Family 81.22:			
4	13.7	16.3	2.6	1	13.1	15.1	2.3
6	15.5	15.7	.2	3	13.9	15.3	1.1
7	15.5	16.0	.5	4	12.8	15.1	2.3
9	15.2	15.5	.3	5	15.7	15.8	.4
11	15.7	15.6	−.1	7	15.8	15.5	−.2
13	15.2	16.0	.8	8	15.5	15.9	.2
14	15.5	15.5	0	9	15.9	15.7	−.5
15	15.4	15.9	.5	10	16.3	16.1	.4
16	15.3	15.6	.3	12	16.5	16.0	−.5
17	16.0	15.8	−.2	14	15.6	16.0	.
18	15.6	15.7	.1	15	15.8	15.8	0
19	15.6	15.5	−.1	17	16.2	15.8	−.4
				19	15.8	15.9	.1
Average	15.2	15.7	.52	20	15.7	15.7	0
				21	16.6	15.9	−.7
				23	15.6	15.7	.1
				Average	15.4	15.7	.28

TABLE 9.—*Values calculated from data in table 8 to show differential effects of leaf rust upon differences of protein content in certain wheat families in 2 years*

Data used	Item	Values for family—			
		54.2	60.4	81.1	81.22
Using all pairs	Plant selections (per family)...number	17	13	19	16
	Mean...percent	0.87	0.52	−0.27	0.28
	Standard deviation	1.00	0.78	1.17	0.84
	Z value	0.87	0.67	0.23	0.33
	Odds	620:1	43:1	5:1	8:1
Omitting series 329 and 368 for 1934 and 1935, respectively	Plant selections (per family)...number	13	11	15	13
	Mean...percent	0.38	0.21	−0.85	−0.09
	Standard deviation	0.50	0.29	0.31	0.33
	Z value	0.75	0.71	2.76	0.28
	Odds	81:1	40:1	+10,000:1	4:1

The two families least injured by leaf rust had a larger protein content, which was of significant value, in 1935 than in 1934. Family 60.4, which showed least leaf deterioration, had a smaller increase than family 54.2. The two families showing greatest damage from leaf rust and greater relative damage in 1935, as compared with the two other families, showed either a decrease of protein content in 1935

or an increase that was not significant. Family 81.22, having the greatest relative reduction in yield and weight per 1,000 kernels in 1935, showed a smaller decrease in protein content in the second year than family 81.1. If families 54.2 and 60.4 are compared as a group with families 81.1 and 81.22 the difference in protein content for the two groups for the 2 years is 0.73 ± 0.17 percent. The odds are thus 370:1 that the wheats showing least injury from leaf rust in 1935 were less affected in the amount of protein laid down in the grain than were other wheats showing greater injury from leaf rust in 1935 as compared with 1934. The leaf-rust readings in families 81.1 and 81.22 were nearly the same for the 2 years, and in both years the readings were moderate. The high temperatures of 1935 in conjunction with the leaf rust present reduced the protein content measurably, but apparently yield, weight per 1,000 kernels, and weight per bushel were more susceptible to the effects of leaf rust than was protein content.

DISCUSSION

Evidence has been presented indicating that a necrotic condition of the wheat leaf induced by leaf rust may influence yield and other characters very decidedly in a season such as 1935 when temperatures are high. The yield deficiency is shown to be aggravated when the necrotic areas are accompanied by leaf rust lesions. When these lesions are present leaf deterioration increases and the pustular effects afford another probable source of injury. Weaver (*9*) describes an experiment conducted by Humphrey with young wheat plants affected by leaf rust in which transpiration was increased 38 percent when less than 1 percent of the transpiration area of the wheat plant was involved by the rust pustules. The area covered by leaf rust in families 81.1 and 81.22 was evidently larger than on the plants studied by Humphrey. It is not possible to draw any close parallel between the two pieces of work except to state that marked results were secured in both instances, in transpiration in the one case and yield in the other. Results secured by Mains (*5*), which are given in table 1, show a distinct loss in yield and kernel weight in the Webster variety when leaf rust inoculation produced only flecking instead of pustulation. The definite losses noted in the present work became evident under conditions of the high 1935 temperatures. There was no way of measuring the damage that might have arisen in 1934.

Chlorosis of the wheat leaf, which often proceeds to further tissue injury, is commonly observed in various wheat varieties but in different degrees. In some cases yellowing apparently results from physiological disturbances, as evidences of infection are not noticeable. Selections from certain crosses seem inclined in this direction. In other cases flecking and the resulting necrosis are obviously due to invasion of rust spores. It is not apparent from the literature that much attention has been paid to leaf chlorosis either as to its cause or its effect upon yield. Probably one reason for this last-named condition is the difficulty of securing quantitative measurements resulting from injury to the green leaf areas. The present study leads to the belief that differences in temperature have a marked influence upon the relationship of such injury to yield and other characters. A rather common opinion, expressed by Richardson (*8*) and others, is that wheat varieties having narrow leaves and scanty foliage give

better returns under dry conditions than do varieties with more ample foliage. This being true, one might presume that with the dry seasonal conditions which prevailed in 1935, and with the families of hybrids discussed in this paper, which have rather well-developed leaf blades, a loss of some of the leaf tissue by necrosis would not influence the character of grain yield. Evidently such was not the case, for a reduction in kernel weight and yield was associated with injury to the leaf.

SUMMARY

By comparing 20 hybrid selections from each of 4 Ceres × (Hope × Florence) families of wheat grown during 1934 and 1935, it was possible to measure the differential effect of leaf rust under two conditions of temperature. The maximum average of leaf rust for any family was only 13 percent.

The family with high resistance to leaf rust and to flecking maintained its yield during 1935, as compared with 1934, in the face of higher temperatures. A second family, resistant to leaf rust but somewhat susceptible to flecking, showed a loss of 16 percent in yield from 1934 to 1935. Two other families, suffering more from leaf rust and from flecking than the two families just mentioned, showed losses of 19 and 28 percent as compared to 1934.

The losses in weight per 1,000 kernels corresponded closely to losses in yield, and this correspondence indicates that the losses in yield were due mainly to reduced size of kernel. Almost no shriveling of the kernel was observed. Weights per bushel were less in 1935, but the decreases here were decidedly smaller than for yield and weight per 1,000 kernels. Heat alone probably affected kernel and bushel weight somewhat adversely.

The two families showing resistance to, or freedom from, leaf rust had a higher protein content in 1935, as compared with 1934, while the two susceptible families showed no increase. The data clearly indicate that the presence of leaf rust reduced the protein content.

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THE INFLUENCE OF COOKING AND CANNING ON THE VITAMIN B AND G CONTENT OF LEAN BEEF AND PORK [1]

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INTRODUCTION

As knowledge of vitamins and their importance in nutrition has broadened, it has become increasingly important to determine, not only the distribution and relative quantities of the several vitamins in raw foodstuffs, but also their vitamin potency as commonly prepared for human or animal consumption. It is well known that such processes as drying, cooking, and canning may alter markedly the vitamin potency of foods, the extent of alteration depending on the processes used and the particular vitamins contained in the food.

Meats have an important place in the American diet. Properly prepared, they furnish protein, energy, certain minerals, notably iron, and vitamins in a palatable and satisfying form. The importance of meat as a source of the vitamin G is well known.

The principal vitamins in lean meat are the antineuritic vitamin B (B_1) and the vitamin G (B_2) complex. Vitamin B is rather easily destroyed by heat, whereas vitamin G is stable, even at fairly high temperatures. Since beef and pork are commonly cooked or canned for human consumption, it seemed worth while to determine what effects, if any, these processes have on their vitamin potency.

PREVIOUS WORK ON VITAMINS B AND G IN MEAT

Comparatively little work had been done on the vitamin content of meats when this project was planned. Most of the work reported had been on raw meats, and involved the vitamin B complex, rather than the separate vitamins B and G.

With pigeons as test animals, Hoagland (*6*)[3] had found the muscle tissues of the mature ox, calf, and lamb relatively poor to fair in vitamin B, when fed in the form of dried-meat preparations. He found also that hog muscle was richer in vitamin B than ox or sheep muscle, 15 percent in the diet being sufficient to protect pigeons from polyneuritis. Baked pork tenderloin was practically as rich in vitamin as the uncooked muscle, but cooked ham had a somewhat lower value than the raw product. Later Hoagland (*7*) reported that dried hog muscle added to a diet of polished rice, as 5, 6.6, and 10 percent of the diet, prevented polyneuritis in pigeons, showing pork to be a good source of vitamin B.

[1] Received for publication Sept. 5, 1935; issued October, 1936. The vitamin terminology used is from the committee report of the American Biological Chemists (*13*)[3]. Paper no. 20, Journal Series, North Dakota Agricultural Experiment Station. The vitamins are also designated according to the English system in paragraph 3.

[2] The authors had the technical assistance of Mrs. Eleanor Bue, Bernice Emmons, and Dorothy Berrigan in feeding the rats and in the tabulation of data, and of L. L. Nesbitt, assistant agricultural chemist, in the preparation of food materials.

[3] Reference is made by number (italic) to Literature Cited, p. 432.

If, as indicated by Seidell (*12*), pigeons do not need vitamin G, but are very sensitive to vitamin B deficiency, then the foregoing work was, in reality, a measure of the relative amounts of the antineuritic factor vitamin B only, and not a measure of vitamin G or the vitamin B complex.

In 1926 Goldberger and his associates (*5*) published results of extensive experiments on both man and animals. In their studies on man, they found that a daily allowance of 200 g of fresh, lean beef, freed from visible fat, gristle, and tendons, when finely chopped and mixed with a little water, seasoned with salt and quickly brought to a boil, gave full protection against pellagra.

Their studies on pellagra in man were supplemented by extensive experiments with albino rats. The tests with rats furnished evidence that the so-called "water-soluble B" consisted of at least two factors. One was the antineuritic heat-labile factor and the other, a heat-stable factor, was probably the same as the pellagra-preventing factor (P-P) found in meat and yeast.

Shortly after the present project was planned, but before work was started, Hoagland (*8*) (1929) published a report of tests with both pigeons and rats. He found that 5 percent of dried lean pork protected pigeons against polyneuritis and loss in weight for 8 weeks and longer. Both fresh and smoked hams were found to be equally good sources of the antineuritic vitamin B. Beef was less potent in the antineuritic vitamin B, since it required 35 to 40 percent of the dried beef in the diet to protect pigeons against polyneuritis for 8 weeks.

In his work with rats, Hoagland did not test for vitamins B and G separately, but measured the combined effect of the "water-soluble vitamins." His experiments indicated that pork was a good source of the water-soluble vitamins, but not so good as brewer's yeast. He states (*8, p. 446*):

> Lean beef contained much less water-soluble B vitamins than lean pork. From 40 to 70 percent of dried fresh beef was required for excellent growth in rats as compared with 15 to 25 per cent of dried lean pork or 5 per cent of dried brewer's yeast.

Later, Hoagland and Snider (*10*) reported work on the relative quantities of growth-promoting vitamin G in beef, pork, lamb, beef spleen, beef liver, pork liver, and beef kidney. On the basis of air-dried, fat-free material, beef, pork, and lamb appeared to contain approximately the same quantities of vitamin G; 15 to 20 percent of either in the diet being sufficient for excellent growth in rats.

BASAL DIET FOR VITAMIN B DETERMINATIONS

After considering a number of commonly used basal diets for vitamin B determinations, it appeared that the basal diets used by Goldberger and his associates (*5*), and by Hoagland (*8*) were essentially alike and satisfactory for this work.

The following basal diet, free from vitamin B, was adopted and found satisfactory:

	Percent
Purified casein	20
Crisco	8
Cornstarch	51
Cod-liver oil	2
McCollum's salt mixture no. 185	4
Autoclaved baker's yeast	15

The casein, a lactic acid-precipitated product, was extracted with dilute acetic acid, essentially according to the recommendations of Steenbock,[4] but as tests showed that the first lot of casein prepared still contained some vitamin B, the method was modified to insure complete extraction. The procedure found satisfactory and adopted was to extract the casein (15 pounds in a large muslin sack) with dilute acetic acid (about 100 cc of glacial acetic acid to 60 liters of water) in a 25-gallon wooden tub for 12 days. The casein was thoroughly drained and the solution changed once a day. Following this period, the casein was washed with distilled water for 4 days. Each day the casein was drained and fresh water added. The extracted casein was then dried in an electric oven at 130° F. and ground to pass through a 20-mesh sieve.

Crisco was purchased on the market as required. The cornstarch was the ordinary household product and was used without further treatment. One lot of cornstarch was extracted with dilute acetic acid similarly to the casein, but as no difference in results was observed, no further extractions were made. The cod-liver oil was a high-grade product of tested potency.

A pure dehydrated bakers' yeast was used throughout. It was treated and autoclaved according to the method of Chase (*2*) and Chase and Sherman (*3*). It was dried in a cool air-drying apparatus (*11*). According to Chase, 15 percent of the dried autoclaved yeast in the basal diet would furnish an adequate amount of vitamin G without vitamin B. This was confirmed in feeding tests by the authors.

Numerous tests indicated that this diet was satisfactory for the quantitative estimation of vitamin B. The negative control rats, on the basal B-free diet alone, became depleted in 13 to 15 days with an average gain of 20 to 25 g, developing polyneuritis, which was followed by death, in 18 to 28 days after depletion. The positive controls fed the B-free diet plus a known source of vitamin B made normal gains over an indefinite period.

BASAL DIET FOR VITAMIN G DETERMINATIONS

In choosing a basal diet for the vitamin G determinations, it appeared that the alcoholic extract of white corn as used by Goldberger and his associates (*5*) and by Hoagland and Snider (*9*) would be satisfactory as a source of vitamin B, free or practically free from vitamin G.

As different investigators had used alcohols of varying strength, it seemed desirable to conduct a number of preliminary tests. These tests confirmed the findings of others in showing that extracts of white corn made either with 80- or 85-percent alcohol, by weight, were potent in vitamin B and free, or practically free, from vitamin G. Extracts made with 95-percent alcohol contained no appreciable amounts of vitamin B.

These tests indicated also that the extract of corn made with 85-percent alcohol, by weight, and dried on cornstarch so that 1 g of extract was equivalent to 4 g of corn, furnished enough vitamin B for normal growth when included as 7 percent of the diet. Feeding the extract at levels of 10 and 13 percent did not increase the rate of gains.

[4] Steenbock, H. Personal communication to authors.

While these tests were in progress, a commercial vitamin B preparation appeared on the market. In this preparation the vitamin extract was dried on cornstarch so that 5 percent in the diet should furnish adequate amounts of vitamin B. However, the results of feeding tests indicated that 5 percent of the preparation in the diet did not always furnish enough vitamin B, but that 10 percent not only furnished adequate amounts but allowed a margin of safety.

Comparative tests showed that 1 g of the commercial vitamin B preparation was equivalent to 1 g of the 85-percent alcohol extract of corn in which 1 g of extract represented 4 g of corn, and that 7 percent of either in the diet furnished enough vitamin B for normal growth. Since the commercial preparation was found satisfactory, and its use simplified the procedure, it was adopted in preference to the alcoholic extract of corn and was employed in all the tests of the meat preparations.

On the basis of these preliminary tests, the basal vitamin G-free diet adopted was the same as the basal vitamin B-free diet except that 10 percent of the commercial vitamin B preparation and 5 percent of added cornstarch replaced the 15 percent of autoclaved yeast in the vitamin B-free diet.

EXPERIMENTAL PROCEDURE

GENERAL METHOD

The general plan followed in this series of experiments, to determine the vitamin B and G potency of raw, cooked, and autoclaved meats, was the usual rat-growth procedure.

Young albino rats 28 to 30 days old, from known parentage, were placed in large cages having wire-screen bottoms, and fed as a group until growth ceased. The basal diet used for feeding was adequate, so far as known, in all food factors except the specific vitamin to be studied.

The preliminary depletion period was followed by an 8-week experimental period, during which the animals were kept in individual cages with wire bottoms, and fed the basal diet with the meat preparations included in varying percentages or in definite amounts as supplements to the basal diet. The rats had free access to distilled water. Weekly records were kept of live weights, food intake, food waste, and symptoms of vitamin deficiency.

At the end of the 8-week experimental period, all surviving animals were etherized and autopsied, and notations were made of both external and internal conditions.

Two series of feeding tests were made for vitamin B in testing the meat preparations for vitamin potency. In the first series, the dried meats were fed as varying percentages of the basal diet. This was to determine approximately how much of the dried meat was required to maintain an average growth rate of 3 g per week over a period of 8 weeks, which is the growth rate suggested by Chase and Sherman (*3*) and Borquin and Sherman (*1*) for the quantitative determination of vitamins B and G.

In the second series, the different meat preparations were fed in definite amounts separately from the basal diet and not as a percentage of the diet. A modification of the paired system of feeding was used, whereby the rats were selected and matched in sets of three, as

nearly as possible, of the same sex, age, and weight. The rats of each set of three were distributed, one each, to the groups fed the raw, cooked, and autoclaved meats. All three rats in the same set were fed a weighed amount of basal diet, restricted to the level of the rat eating the least, plus supplements of the raw- cooked, and autoclaved meat preparations, respectively. Although this adaptation of the paired feeding method has the disadvantage of keeping the food intake of some rats below the amount they would otherwise eat, it has the distinct advantage of limiting the errors in the comparison of the three meat preparations. Differences in growth of the rats on raw, cooked, and autoclaved meats can be attributed to differences in the quantitative vitamin potency of the meats, if all variables in food intake are controlled.

THE MEAT PREPARATIONS

The meats used for testing were fresh hams and fresh beef round. Enough meat was prepared at one time to carry through an entire unit of the experiment. No attempt was made to determine the origin of the meat. Two lots of both beef and pork were tested. The lean meat was separated by hand from the visible fat and connective tissue and was then ground three times through a meat grinder of the plate type and thoroughly mixed. The ground meat was divided into three portions and treated as follows:

(1) Raw meat: Dried and finely ground.

(2) Cooked meat: The ground meat was heated and stirred in a double boiler until it reached a constant temperature of approximately 90° C., then dried and finely ground.

(3) Autoclaved meat: The raw ground meat was sealed in no. 2 tin cans without water and processed for 70 minutes at 10 pounds pressure in a pressure cooker or autoclave.[5] The cans were packed as they would be packed for home canning, and not evacuated. After processing, the cans were opened and the meat was dried and finely ground. No determinations were made of the temperature attained by the meat inside the cans.

All meat samples were air-dried in glass plates on a cool air-drying apparatus designed and described by Hopper (*11*). The meat juices which escaped from the meat upon cooking were dried with the meat.

The temperature of the meat samples during drying usually ranged from 9° to 14° C. In 20 to 22 hours the meat was sufficiently dry for grinding. It was then finely ground and further dried in vacuo over sulphuric acid. The dried meats were placed in half-pint glass fruit jars, the rubbers moistened with glycerin, and the covers clamped in place. The fruit jars were then placed in vacuum desiccators and thoroughly evacuated. When the desiccators were opened, the covers automatically clamped tight, thus sealing the dried meat under partial vacuum. The meat preparations so sealed were kept in an electric refrigerator until used. At the low temperature and with the speed at which the drying was accomplished, it is believed that the minimum oxidation took place and that storage under vacuum prevented deterioration upon standing.

When the jars were opened for use, the raw dry meat was found to have retained its deep reddish-brown color and the odor of raw meat.

[5] The processing time and pressure were erroneously stated as 60 minutes and 15 pounds, respectively, in the preliminary report 4.

The cooked and autoclaved meats had a brownish-gray color and the odor of a good roast. The flavor of the different preparations was retained in the dried product.

CHEMICAL ANALYSES OF MEATS

Analyses were made of the dried pork and beef preparations to determine the water, protein, and fat content. The average composition of all samples, on a water-free basis, is given in table 1.

TABLE 1.—*Average percentages of protein and fat in water-free meat preparations, raw, cooked, and autoclaved*

Meat and condition	Protein	Fat
Pork:	*Percent*	*Percent*
Raw	84.98	10.90
Cooked	85.98	10.34
Autoclaved	86.35	10.38
Beef:		
Raw	92.60	3.85
Cooked	91.75	3.74
Autoclaved	91.64	3.64

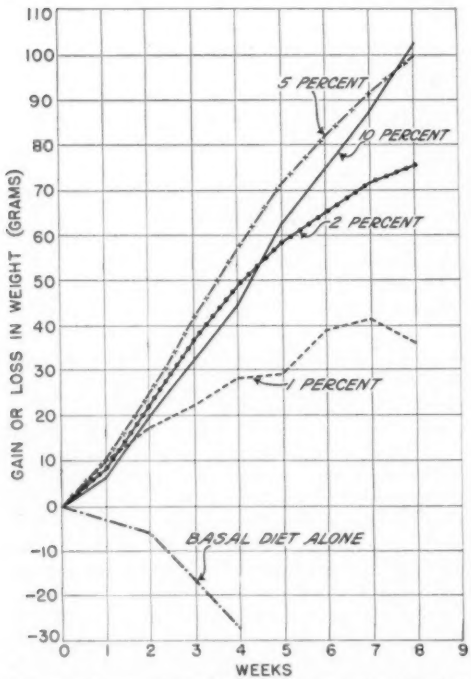

FIGURE 1.—Composite growth curves of rats on the basal diet alone and on the basal diet supplemented with different percentages of dried raw lean pork to provide vitamin B. Two rats were fed the basal diet alone, six received 1 percent, six received 2 percent, nine received 5 percent, and two received 10 percent of pork in their diet. Series 1.

Through a misunderstanding, the water content of the fresh meats in relation to the dried preparations was not determined on all preparations, but determinations on several of the different lots indicated that 1 part of dried product was equal to 3.3 to 4 parts, by weight, of the fresh meat.

VITAMIN B IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN PORK

SERIES 1: DRIED PORK FED AS A PERCENTAGE OF THE DIET

To determine at what level the dried lean pork should be fed to produce the desired gain of 3 g per week during the test period, four groups of rats were fed the dried raw, cooked, and autoclaved pork as 1, 2½, 5, and 10 percent of the total food intake. Adjustments were made in the protein and fat to maintain approximately a constant level of 20 percent of protein and 8 percent of fat in the diet. These adjustments for protein and fat were not made in later experiments

where the supplements were fed separately from the basal diet. Wherever the meat preparations were fed as a percentage of the diet, they replaced equal amounts of starch in the basal diet.

The composite growth curves for the groups on the raw dried pork are shown in figure 1.

It is worthy of note that the group on 10 percent of pork made no better gains than the group on 5 percent. The rats on 2 percent of pork gained approximately twice as much as those on 1 percent, but considerably less than those on 5 percent. All the rats fed the different levels of uncooked pork survived the entire 56-day period without any definite polyneuritic symptoms.

Table 2 gives the average weights, gains, and food consumption of all the different groups of rats.

TABLE 2.—*Average weights, gains in weight, and food consumption of groups of rats fed various percentages of dried lean pork, raw, cooked, or autoclaved, as a source of vitamin B in series 1*

Percentage of dried pork in diet	Rats	Average time on diet	Initial weight	Final weight	Total gain	Average per week				Gain per gram of pork fed	Gain per gram of total food
						Gain	Basal diet	Pork fed	Total food		
	No.	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1, raw	6	56	85.5	122.0	36.5	4.6	46.1	0.47	46.6	9.79	0.099
1, cooked	[1] 5	56	85.8	118.4	32.6	4.1	46.1	.47	46.5	8.72	.088
1, autoclaved	[1] 5	56	92.0	127.2	35.2	4.4	47.3	.48	47.7	9.17	.092
1, cooked	[2] 6	52.5	87.2	108.3	21.1	2.8	43.1	.44	43.5	6.36	.064
1, autoclaved	[2] 6	52.7	91.3	114.0	22.7	3.0	43.2	.44	43.6	6.82	.069
2, raw	6	56	78.8	154.5	75.6	9.5	51.1	1.04	52.2	9.13	.182
2, cooked	6	56	76.8	150.8	74.0	9.2	51.6	1.05	52.7	8.76	.175
2, autoclaved	6	56	78.5	151.2	72.7	9.1	50.8	1.04	51.9	8.75	.175
5, raw	9	56	87.6	187.7	100.1	12.5	54.8	2.89	57.7	4.33	.217
5, cooked	8	56	80.5	180.6	100.1	12.5	55.9	2.94	58.8	4.25	.213
5, autoclaved	6	56	79.0	170.3	91.3	11.4	54.6	2.88	57.5	3.96	.198
10, raw	2	56	114.5	218.0	103.5	12.9	57.5	6.39	63.8	2.02	.202
10, cooked	2	56	74.0	176.5	102.5	12.8	53.0	5.89	58.9	2.17	.217

[1] Average for the rats that completed the full 56-day period. 1 rat in each group on the 1-percent level of cooked and autoclaved pork died of polyneuritis.
[2] Average for all rats on test.

The data of table 2 show no definite destruction of vitamin B as a result of cooking or autoclaving the pork. However, on 1 percent of dried pork in the diet, one rat in each group fed the cooked and autoclaved pork died of polyneuritis near the middle of the 56-day period. One or two other rats in each of these two groups had rough coats and showed signs of nervousness. This was interpreted as possibly due to a partial destruction of vitamin B.

The average weekly intake of pork on the 1-percent level was approximately 0.45 g, and this amount was chosen for the tests of series 2 on pork.

SERIES 2: DRIED PORK FED SEPARATELY FROM THE BASAL DIET

Eight sets of three matched rats each were fed weighed amounts of the basal diet plus 0.45 g weekly of the dried raw, cooked, or autoclaved pork. To facilitate the weighing of these small amounts of pork, it was mixed with cornstarch, so that 1.5 g of the mixture represented 0.45 g of meat. The weekly portion of supplement was weighed out at the first of the week and fed in three approximately equal feedings. The comparative gains of the rats on the different preparations are shown in figure 2.

It appears from figure 2 that cooking destroyed some of the vitamin B and that autoclaving destroyed more, but not all, of the vitamin.

All of the 24 rats fed in series 2 survived the full experimental period and none showed any definite symptoms of vitamin B deficiency. As a rule, the rats on the autoclaved pork ate the least of the basal diet. Since the food intake in each set of three matched rats was restricted to the rat eating the least, the food intake of the rats on the raw and cooked pork was thereby largely restricted to the level of those fed the autoclaved pork. The principal data for series 2 are given in table 3.

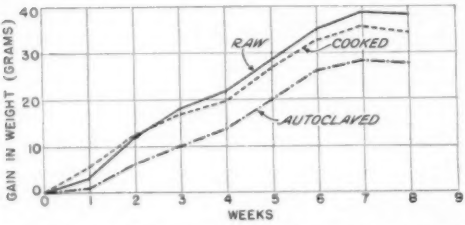

FIGURE 2.—Composite growth curves of three groups of eight rats each receiving 0.45 g of raw, cooked, and autoclaved canned lean pork, respectively, per rat weekly, fed separately from the basal diet in three equal portions to provide vitamin B. Series 2.

Table 3 shows that the food intake was virtually identical on the raw and cooked pork, but averaged about 2 g less per week on the autoclaved pork. The smaller food intake of the rats fed the autoclaved pork was probably due to a partial destruction of the appetite-stimulating vitamin B or some unknown factor. The smaller gains made by the rats on the autoclaved pork are only in part accounted for by the smaller food intake.

Since the total food intake was more accurately controlled in series 2, more weight is given to the results of these tests than to those in which the meat was fed as a percentage of the diet.

TABLE 3.—*Average weights, gains in weight, and food consumption of groups of eight rats fed three equal portions of dried pork, raw, cooked, or autoclaved, separately from their basal diet and at a level of 0.45 g per rat per week, as a source of vitamin B, in series 2 tests lasting 56 days*

Kind of pork fed	Initial weight	Final weight	Total gain	Average per week				Gain per gram of pork fed	Gain per gram of total food
				Gain	Basal diet	Pork fed	Total food		
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Raw	77.4	115.0	37.6	4.7	39.3	0.45	39.8	10.44	0.118
Cooked	77.4	110.8	33.4	4.2	39.7	.45	40.2	9.33	.104
Autoclaved	79.3	106.9	27.6	3.5	37.5	.45	38.0	7.78	.092

VITAMIN B IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN BEEF

SERIES 1: DRIED BEEF FED AS A PERCENTAGE OF THE DIET

Thirty-seven rats were fed the vitamin B-free basal diet until depleted and then separated into groups and fed the dried raw lean beef at levels of 2, 5, 7, 10, and 15 percent of the diet to determine the amount of meat required to produce the desired gain of 3 g per week. No cooked or autoclaved beef was fed in this series.

The composite curves of gains or losses by weeks are shown in figure 3, based on the averages of the rats in each group that survived the full 8-week period.

The response of the individual rats to the different levels of beef in the diet varied considerably, especially in some groups, in spite of the fact that the rats were from the same stock and had received the same treatment. These variations are not apparent from the graphs or tables, but are significant in the interpretation of the data and, consequently, are given in some detail.

All the rats fed 2 and 5 percent of dried beef in the diet developed polyneuritis, which resulted in the death of one rat in the 2-percent group after 26 days, and two rats in the 5-percent group. One of these rats died after 8 and the other after 27 days on the test. The remaining rats in each group recovered rapidly when fed yeast.

Three of the nine rats that were started on the 7-percent level of dried beef died after 11, 16, and 19 days, respectively, on the diet. One of the remaining six was fed yeast from the fifth week, but it made only fair recovery. The five rats that survived the full 8-week period made small, but rather uniform, gains during the first 5 weeks, followed by accelerated gains during the last 3 weeks. No explanation for the accelerated gains is apparent.

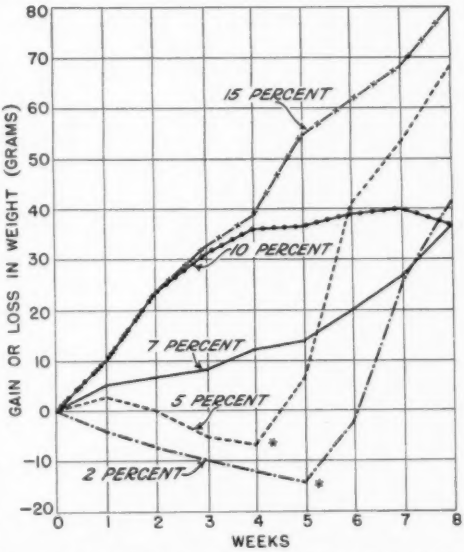

FIGURE 3.—Composite growth curves of rats fed the basal diet supplemented with different percentages of dried raw lean beef to provide vitamin B. Two rats received 2 percent, three received 5 percent, five received 7 percent, seven received 10 percent, and five received 15 percent of beef in their diet. The rats fed at the 2- and 5-percent levels were near death when yeast was added to their diets as indicated by the asterisk (*) on the graph.

Three of the twelve rats started on the 10-percent level of dried beef died after 14, 23, and 45 days, respectively, and two rats after reaching a weakened condition, improved markedly when fed yeast. The remaining seven rats, on 10 percent of beef, made virtually identical gains with those on 15 percent, during the first 4 weeks of the test period, but, as a group, they made practically no gains during the last 4 weeks. An inspection of the individual data shows that, for no apparent reason, four of the seven rats gained more or less consistently throughout the test period, reaching an average weight of 126 g as compared to 108 g reached by the five rats on 7 percent of beef and 145 g reached by the five rats on 15 percent of beef. One of the three remaining rats made only a small gain and the other two lost weight during the last half of the test period, thus making the average weight of these three only 74 g at the end of the test.

Six rats were placed on the 15-percent beef diet, but one died at 31 days, leaving five that completed the full period, with fairly steady and uniform gains throughout.

Table 4 gives a summary of the average weights, gains or losses, and food consumption of the rats at the different levels of beef feeding.

TABLE 4.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed various percentages of dried raw beef as a source of vitamin B in series 1*

Raw beef in diet (percent)	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain or loss per gram of beef	Gain or loss per gram of total food
						Gain or loss	Basal diet	Beef fed	Total food		
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
2	[1] 3	29.7	73.0	55.0	−18.0	−4.3	29.0	0.6	29.6	−7.17	−0.145
5	[2] 7	23.9	63.4	58.4	−5.0	−1.9	32.2	1.7	33.9	−1.12	−.056
7	[3] 5	56.0	70.0	107.6	37.6	4.7	42.3	3.2	45.5	1.47	.103
10	[4] 7	56.0	65.9	102.4	36.5	4.6	39.6	4.4	44.0	1.05	.105
15	[5] 5	56.0	65.5	145.0	79.4	9.9	44.0	7.8	51.8	1.27	.191

[1] Average of all rats in group up to time of feeding yeast; 1 of the 3 rats died before yeast was fed.
[2] Average of all rats in group up to time of feeding yeast; 2 of the 7 rats died before yeast was fed.
[3] 9 rats started; 3 died and 1 was fed yeast; averages are for remaining 5 rats.
[4] 12 rats started; 3 died and 2 were fed yeast; averages are for remaining 7 rats.
[5] 6 rats were started but 1 died; averages are for remaining 5 rats.

From the results obtained, it was difficult to determine how much beef would be required to furnish enough vitamin B to maintain an average gain of 3 g per week, but, apparently, 7 percent in the diet, or 3.2 g of dried beef per week, was about the lower limit.

SERIES 2: DRIED BEEF FED SEPARATELY FROM THE BASAL DIET

Sets of three matched rats each were fed on equal weighed portions of dried raw, cooked, and autoclaved beef, fed separately from the basal diet to insure more uniform consumption of the beef. The basal food intake was also kept as uniform as possible, but it was found that the rats on the autoclaved beef almost invariably decreased their food intake as the period progressed. Whenever a rat on the autoclaved beef ate less than 3 g of food per day, it was dropped from the set and the food intake equalized between the rats on the raw and cooked beef. All the rats relished the raw and cooked beef, but those on the autoclaved beef showed a consistent dislike for it. Some of the autoclaved beef was frequently left uneaten or wasted from the food cup. It was, therefore, impossible to keep the food intake alike for all the rats.

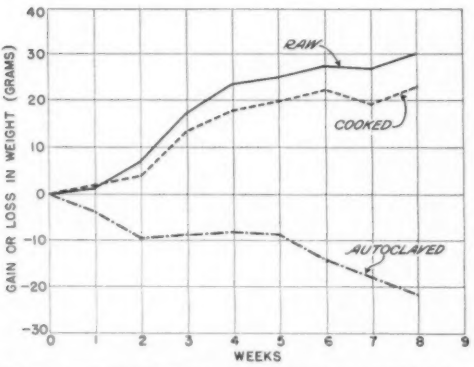

FIGURE 4.—Composite growth curves of two groups of eight and one group of nine rats receiving 4.5 g of raw, cooked, and autoclaved canned lean beef, respectively, per rat weekly, fed separately from the basal diet in three equal portions to provide vitamin B. Series 2.

No such difficulty existed with the pork, possibly due to the fact that the pork was fed in small amounts (less than 0.5 g per week) mixed with cornstarch, or to the larger amounts of vitamin B contained in the autoclaved pork as compared to the beef, or to a loss of the appetite-stimulating factor, vitamin B. The last-mentioned seems the most likely explanation, for when adequate amounts of

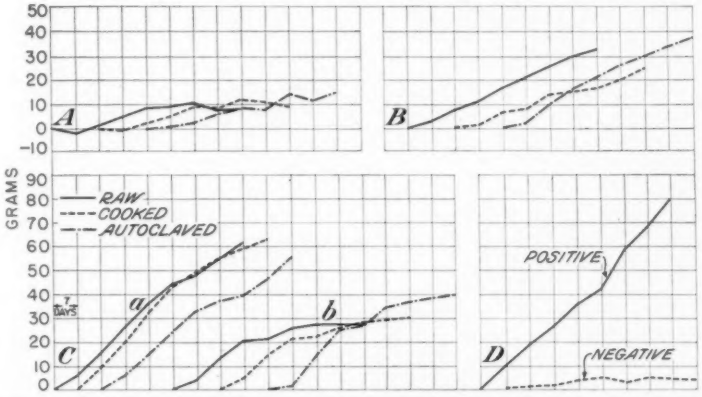

FIGURE 5.—Composite growth curves of rats receiving various quantities of dried pork added separately to their diets to provide vitamin G: *A*, three groups of eight rats each, fed 1 g of pork per rat per week; *B*, three groups of five rats each, fed 1.5 g of pork per rat per week; *C*, three groups of six rats each in series 1 (*a*) and three rats each in series 2 (*b*), fed 2 g pork per rat per week; *D*, groups of four rats on positive and six on negative control diet.

vitamin B were furnished, as in testing for vitamin G, the rats ate the autoclaved beef readily.

Because of the results of series 1, levels of 4, 4.5, and 6 g of dried beef weekly were chosen for series 2 in testing the relative potency of the three preparations for vitamin B.

The composite weight curves of the rats fed 4.5 g of beef per week are shown in figure 4.

TABLE 5.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed three equal portions of dried beef, raw, cooked, or autoclaved, separately from their basal diet and at levels of 4, 4.5, and 6 g per rat per week, as a source of vitamin B in series 2*

Amounts and kind of beef fed weekly	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain or loss per gram of beef fed	Gain or loss per gram of total food
						Gain or loss	Basal diet	Beef fed	Total food		
	Number	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
4 g:											
Raw	5	56	89.0	112.0	23.0	2.9	32.5	4.0	36.5	0.73	0.079
Cooked	5	56	88.2	118.4	30.2	3.8	33.4	4.0	37.4	.95	.102
Autoclaved	5	39	87.6	60.6	−27.0	−5.7	21.2	2.7	23.9	−2.11	−.238
4.5 g:											
Raw	8	54.3	80.9	111.0	30.1	3.9	33.3	4.5	37.8	.87	.103
Cooked	8	54.3	81.4	104.4	23.0	3.0	31.9	4.5	36.4	.67	.082
Autoclaved	9	50.0	84.3	67.3	−17.0	−3.8	23.1	3.9	27.0	−.97	−.141
6 g:											
Raw	2	56.0	72.5	118.5	46.0	5.8	35.2	6.0	41.2	.97	.141
Cooked	2	56.0	69.5	112.5	43.0	5.4	33.7	6.0	39.7	.90	.136
Autoclaved	2	50.5	71.0	57.0	−14.0	−2.1	20.9	4.2	25.1	−.50	−.084

Figure 4 shows that the rats on the cooked beef did not gain as well as those on the raw beef, and also that the ones on the autoclaved beef lost weight from the start. The average results for all the groups at the different levels of beef feeding are shown in table 5.

The small average weekly gains made by the rats on 4 g of dried raw beef per week are mainly due to the fact that one rat wasted food and lost weight; the other four averaged 3.7 g gain per week, which is practically the same as the gains made by the rats on the cooked beef. Of the five rats on the autoclaved beef, one made a small gain of 3 g in 8 weeks; the other four lost from 32 to 38 g.

Of the eight rats fed 4.5 g of raw beef weekly, one lost 2 g, but the others gained from 28 to 40 g each. Similarly, one of the rats on the cooked beef lost 5 g, while the others gained from 9 to 37 g. If the ones that lost weight are omitted, the average weekly gains per rat are 4.5 g on the raw, and 3.5 g on the cooked beef.

All rats fed 6 g of raw and cooked beef weekly survived the entire 8-week period and made larger average weekly gains than the groups fed at the lower levels. Both of the rats fed the autoclaved beef lost weight. One of the rats died at the end of 45 days on the diet.

With one or two exceptions on the 4- and 4.5-g levels, all the rats on the autoclaved beef lost weight, but evidently the autoclaved beef still contained some vitamin B, since the losses in weight decreased and the survival period increased in the successive groups with the increase in level of beef feeding.

UNITS OF VITAMIN B IN DRIED LEAN PORK AND BEEF

The units of vitamin B in the dried lean pork and beef, computed according to the method of Chase and Sherman (*3*), are presented in table 6.

UNITS OF VITAMIN B PER GRAM OF DRIED LEAN PORK

The units of vitamin B in pork, as given in table 6, were computed only at the 1- and 2-percent levels and 0.45 g weekly, as the gains on the 5- and 10-percent levels were too large to furnish a reliable measure.

In series 1, where the pork was fed as a percentage of the diet, there is no clear-cut or definite indication of a destruction of vitamin B as a result of either cooking or autoclaving. However, in series 2, where the pork was fed separately from the basal diet, and where the food was equalized more closely in the different sets of three, there appears to be a rather definite indication that some of the vitamin was destroyed as a result of cooking and autoclaving. From the values found in series 2 alone, it appears that approximately 10 percent of vitamin B was destroyed as a result of cooking and 25 percent as a result of autoclaving the pork. Slightly different results are obtained when the rats on the 1-percent level of pork are included. The weighted average for the rats fed at the 1-percent level of dried pork and those fed 0.45 g per week, separately from the basal diet, is 24 units per gram of dried raw pork. This is equivalent to approximately 7 units per gram of fresh pork. For the cooked pork, the weighted average is 21 units and for the autoclaved pork, it is 19 units per gram of dried product.

TABLE 6.—*Units of vitamin B in variously treated dried pork and beef, as computed from the data by the method of Chase and Sherman*

PORK

Series, treatment of meat used, and quantity fed	Rats	Average time on diet	Average weekly gains or losses in weight	Vitamin B per gram of meat
Series 1:	*Number*	*Days*	*Grams*	*Units*
1 percent, raw	[1] 6	56.0	4.6	22.8
1 percent, cooked	[2] 5	56.0	4.1	20.5
1 percent, autoclaved	[2] 5	56.0	4.4	21.2
1 percent, cooked	[1] 6	52.5	2.8	14.8
1 percent, autoclaved	[1] 6	52.7	3.0	15.9
2 percent, raw	6	56.0	9.5	21.2
2 percent, cooked	6	56.0	9.2	20.2
2 percent, autoclaved	6	56.0	9.1	20.3
Series 2:				
0.45 g, raw, weekly	8	56.0	4.7	24.5
0.45 g, cooked, weekly	8	56.0	4.2	21.9
0.45 g, autoclaved, weekly	8	56.0	3.5	18.2

BEEF

Series 1:				
7 percent, raw	[3] 5	56.0	4.7	3.4
10 percent, raw	[4] 7	56.0	4.6	2.4
15 percent, raw	[3] 5	56.0	9.9	3.0
Series 2:				
4.0 g, raw, weekly	5	56.0	2.9	1.7
4.0 g, cooked, weekly	5	56.0	3.8	2.2
4.0 g, autoclaved, weekly	5	39.0	−5.7	
4.0 g, raw, weekly	[5] 4	56.0	3.7	2.2
4.5 g, raw, weekly	8	54.3	3.9	2.0
4.5 g, cooked, weekly	8	54.3	3.0	1.6
4.5 g, autoclaved, weekly	9	50.0	−3.8	
4.5 g, raw, weekly	[5] 7	56.0	4.5	2.3
4.5 g, cooked, weekly	[5] 7	56.0	3.5	1.8
6.0 g, raw, weekly	2	56.0	5.8	2.3
6.0 g, cooked, weekly	2	56.0	5.4	2.1
6.0 g, autoclaved, weekly	2	50.5	−2.1	

[1] Averages for all rats in the group.
[2] Average, omitting 1 rat that died in each group.
[3] Average of 5 rats out of 9 that lived through entire period without modification of diet.
[4] Average of 7 rats out of 12 that lived through entire period without modification of diet.
[5] Average, omitting 1 rat in each group that lost weight.

UNITS OF VITAMIN B PER GRAM OF DRIED LEAN BEEF

The units per gram of dried beef in series 1 are slightly higher than the units as determined in series 2. In series 2, at the level of 4 g of dried beef per week, there is no definite indication of a destruction of vitamin B, but at the 4.5-g level there appears to be a destruction of vitamin B as a result of cooking. At the 6-g level, there is also no definite indication of a destruction of vitamin B as a result of cooking, but at all three levels, there is definite indication that autoclaving destroyed at least a part of the vitamin contained in the beef. The dried lean raw beef contained an average of about 2.5 units per gram, which is equivalent to 0.6 unit per gram of fresh beef. From this it is evident that the lean beef contained only about one-tenth as much vitamin B as the pork.

It is not possible from the data to explain why autoclaving destroyed the vitamin B more completely in the lean beef than in the lean pork. The data of table 1 show that the lean pork contained about three times as much fat as the lean beef, and it is suggested that the fat may possibly have aided in protecting the vitamin from destruction.

VITAMIN G IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN PORK

Pork was fed at levels of 1, 1.5, and 2 g per week, as preliminary tests had shown, these levels to be suitable for testing for vitamin G. The pork for the entire week was weighed out in advance and fed as a supplement separately from the basal diet in approximately three equal portions during the week.

Composite growth curves for the different groups are shown in figure 5, and a summary of the data by groups, including the controls, is given in table 7.

TABLE 7.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed dried pork, raw, cooked, or autoclaved, separately from their basal diets and at levels of 1, 1.5, and 2 g per rat per week, as a source of vitamin G*

Amounts and treatment of pork fed weekly for period and series indicated	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain per gram of pork fed	Gain or loss per gram of total food
						Gain or loss	Basal diet	Pork fed	Total food		
1 g, September to November 1932:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Raw	8	56	67.9	75.9	8.0	1.0	33.7	1.0	34.7	1.00	0.029
Cooked	8	54	66.1	75.7	9.6	1.2	33.6	1.0	34.6	1.20	.034
Autoclaved	8	56	67.5	81.5	14.0	1.7	33.1	1.0	34.1	1.70	.050
Negative controls	3	56	50.3	73.3	23.0	2.9	33.3	.0	33.3	-------	.087
Positive controls	2	56	53.5	117.0	63.5	8.0	43.6	1.0	44.6	8.00	.179
1.5 g, April to July 1933:											
Raw	5	56	38.8	70.8	32.0	4.0	30.6	1.5	32.1	2.67	.125
Cooked	5	56	38.6	62.2	23.6	3.0	27.7	1.5	29.2	2.00	.103
Autoclaved	5	56	38.2	75.0	36.8	4.6	30.9	1.5	32.4	3.07	.142
Negative control	1	56	57.0	69.0	12.0	1.5	29.8	.0	29.8	-------	.050
2 g, series 1, June to August 1932:											
Raw	6	56	60.1	121.5	61.3	7.7	37.8	2.0	39.8	3.85	.193
Cooked	6	56	59.3	121.8	62.5	7.8	38.6	2.0	40.6	3.90	.192
Autoclaved	6	56	60.5	116.0	55.5	6.9	36.4	2.0	38.4	3.45	.180
Negative controls	2	56	56.0	55.5	−.5	−.1	26.6	.0	26.6	-------	−.004
Positive controls	2	56	45.0	139.5	94.5	11.8	38.2	3.5	41.7	3.37	.283
2 g, series 2, July to September 1933:											
Raw	3	56	63.3	90.6	27.3	3.4	29.2	2.0	31.2	1.70	.109
Cooked	3	56	63.0	93.2	30.3	3.8	31.1	2.0	33.1	1.90	.115
Autoclaved	3	56	63.0	103.0	40.0	5.0	31.2	2.0	33.2	2.50	.151
Negative control	1	56	70.0	75.0	5.0	.6	32.7	.0	32.7	-------	.018

It was not possible to conduct all the feeding trials at the different levels simultaneously. Series 1 at the level of 2 g of dried pork weekly was carried out during the first summer, followed by the group on 1 g of pork weekly during the fall of the same year. The following spring the tests at the 1.5-g level were made, followed by series 2 on the 2-g level during the summer.

The data of table 7 show that 1 g of the dried pork preparations failed to support a unit gain of 3 g per week, and that 1.5 g gave slightly more than a unit rate of gain per week. The gains on 2 g of pork in series 1 were nearly twice as great as the gains on 1.5 g, but in series 2 at the same level of pork as in series 1, the gains were similar to those on the 1.5 g of pork. At first it might appear that a loss in vitamin potency had occurred between the tests of series 1 the first summer, and series 2 the following summer; but this seems unlikely

since the tests at the 1.5-g level were made just prior to the tests of series 2. Moreover, no decrease in potency due to storage was observed in the beef under similar circumstances.

The larger food intake by the rats in series 1 as compared to series 2 may explain in part the differences in the gains of the rats in the two series, but it does not explain why the rats on 1.5 g of pork made virtually the same gains as those on 2 g in series 2 where the food intake was practically the same. Possibly differences in the state of depletion of the rats at the start was a factor.

In spite of some inconsistencies in the gains on the raw, cooked, and autoclaved pork in the different groups, and on the different levels of intake, the data fail to show any destruction of vitamin G in the pork when cooked or autoclaved by the methods here used.

VITAMIN G IN DRIED RAW, COOKED, AND AUTOCLAVED LEAN BEEF

The work of Hoagland and Snider (*10*) had indicated that pork and beef have about the same vitamin G potency. With this in

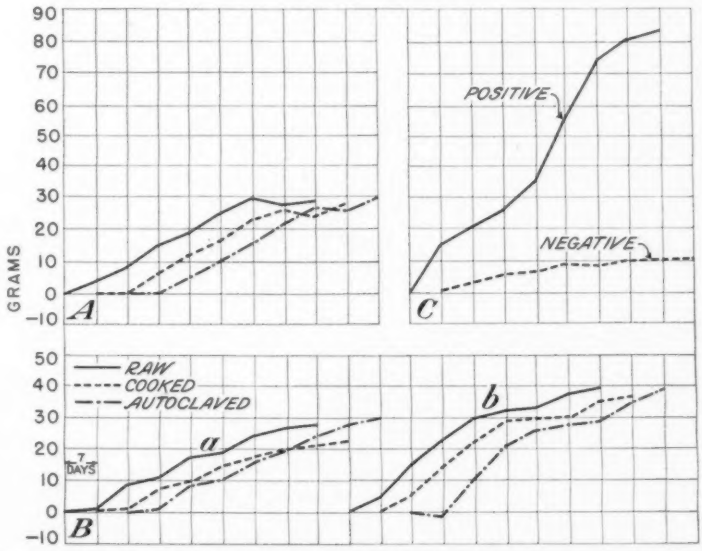

FIGURE 6.—Composite growth curves of rats receiving dried lean beef added separately to their diets to provide vitamin G: *A*, Three groups of 10 rats each fed 1.5 g of beef per rat per week; *B*, three groups of 10 rats each in series 1 (*a*) and 3 rats each in series 2 (*b*) fed 2 g of beef per rat per week; *C*, 1 rat on positive and group of 6 rats on negative control diet

mind the writers fed dried beef only at levels of 1.5 and 2 g per week, as 1 g of pork had been found inadequate.

The composite growth curves of the rats fed in these tests are shown in figure 6, and a summary of the gains and food intake is given in table 8.

TABLE 8.—*Average weights, gains or losses in weight, and food consumption of groups of rats fed dried beef, raw, cooked, or autoclaved, separately from their basal diets and at levels of 1.5 and 2 g per rat per week, as a source of vitamin G*

Amounts and treatment of beef fed weekly for period and series indicated	Rats	Average time on diet	Initial weight	Final weight	Total gain or loss	Average per week				Gain per gram of beef fed	Gain or loss per gram of total food
						Gain or loss	Basal diet	Beef fed	Total food		
1.5 g, March to May 1933:	*Number*	*Days*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Raw	10	56	52.0	80.6	28.6	3.6	33.9	1.5	35.4	2.40	0.102
Cooked	10	56	52.4	79.5	27.1	3.4	34.0	1.5	35.5	2.27	.096
Autoclaved	10	56	52.3	81.5	29.2	3.7	33.7	1.5	35.2	2.47	.105
Negative controls	4	56	52.5	65.0	12.5	1.6	31.0	.0	31.0	-------	.052
2 g, series 1, December to February 1932–33:											
Raw	10	56	63.0	90.1	27.1	3.4	34.4	2.0	36.4	1.70	.093
Cooked	10	56	61.7	84.2	22.5	2.8	33.9	2.0	35.9	1.40	.078
Autoclaved	10	56	61.4	90.2	28.8	3.6	33.8	2.0	35.8	1.80	.101
Negative controls	1	56	34.0	45.0	11.0	1.4	26.5	.0	26.5	-------	.053
Positive controls	1	56	47.0	130.0	83.0	10.4	51.1	2.0	53.1	5.20	.196
2g, series 2, July to September 1933:											
Raw	3	56	59.7	98.7	39.0	4.9	32.7	2.0	34.7	2.45	.141
Cooked	3	56	61.0	97.3	36.3	4.5	32.7	2.0	34.7	2.25	.130
Autoclaved	3	56	62.0	100.3	38.3	4.8	32.2	2.0	34.2	2.40	.140
Negative control	1	56	44.0	42.0	−2.0	−.3	21.3	.0	21.3	-------	−.014

The 10 sets of 3 matched rats each fed 1.5 g of dried beef per week separately from the basal diet (table 8) were fed simultaneously with the 5 sets of 3 rats on 1.5 g of dried pork (table 7). The data show that the rats on the raw, cooked, and autoclaved beef gained 3.6, 3.4, and 3.7 g, respectively, as compared to gains of 4.0, 3.0, and 4.6 g by the rats on the pork. These results indicate that the vitamin G potency of the beef and pork preparations was practically the same.

Although there were considerable variations in the rates of gain among the groups, the results indicate no loss of vitamin G potency as a result of either cooking or autoclaving.

UNITS OF VITAMIN G IN DRIED LEAN PORK AND BEEF

The units of vitamin G in the several feeding periods and at the different levels of feeding have been computed according to the method of Borquin and Sherman (*1*), and are given in table 9.

The units of vitamin G per gram of dried pork and beef in table 9 vary considerably between different levels of feeding and between series on the same level, but a comparison of the units per gram of meat fails to show any definite destruction of vitamin G as a result of cooking or autoclaving. Using the data from all the rats fed at the 1.5 and 2 g levels, the weighted average units of vitamin G per gram of dried meat are: 6.9 for the raw, 6.5 for the cooked, and 7.3 for the autoclaved pork, as compared to 4.9 for the raw, 4.4 for the cooked, and 5.1 for the autoclaved beef.

TABLE 9.—*Units of vitamin G in variously treated dried pork and beef as computed from the data by the method of Borquin and Sherman*

PORK

Series, treatment of meat used, and quantity fed weekly	Rats	Average time on diet	Average weekly gains in weight	Vitamin G per gram of meat
	Number	*Days*	*Grams*	*Units*
1 g, raw	8	56	1.0	2.3
1 g, cooked	8	54	1.2	2.8
1 g, autoclaved	8	56	1.7	4.0
1.5 g, raw	5	56	4.0	6.2
1.5 g, cooked	5	56	3.0	4.7
1.5 g, autoclaved	5	56	4.6	7.2
Series 1:				
2 g, raw	6	56	7.7	9.0
2 g, cooked	6	56	7.8	9.1
2 g, autoclaved	6	56	6.9	8.1
Series 2:				
2 g, raw	3	56	3.4	4.0
2 g, cooked	3	56	3.8	4.4
2 g, autoclaved	3	56	5.0	5.8

BEEF

1.5 g, raw	10	56	3.6	5.6
1.5 g, cooked	10	56	3.4	5.3
1.5 g, autoclaved	10	56	3.7	5.8
Series 1:				
2 g, raw	10	56	3.4	4.0
2 g, cooked	10	56	2.8	3.3
2 g, autoclaved	10	56	3.6	4.2
Series 2:				
2 g, raw	3	56	4.9	5.7
2 g, cooked	3	56	4.5	5.2
2 g, autoclaved	3	56	4.8	5.6

SUMMARY OF RESULTS

The main facts brought out in these studies on pork and beef as are follows:

(1) The dried raw lean pork used in the tests was a good source of vitamin B, containing about 24 units per gram of dried material. This is equivalent to about 7 units per gram of fresh pork. The dried raw lean beef contained 2.5 units of vitamin B per gram, equivalent to 0.6 unit per gram of fresh beef. On this basis the fresh lean beef contained less than one-tenth as much vitamin B as the pork.

(2) The lean pork cooked at a temperature not exceeding 90° C. contained 21 units of vitamin B per gram of dried material, representing a loss of 12 percent in potency. Lean beef cooked in the same way contained 2 units per gram of dried material, representing a loss in potency of 20 percent.

(3) Heating the pork in a steam autoclave or pressure cooker for 70 minutes at 10 pounds' pressure destroyed more of the vitamin B than did cooking. The autoclaved pork had a value of about 19 units per gram of dried material, representing a loss in potency of approximately 21 percent. Under similar treatment the vitamin B in the beef was almost completely destroyed.

(4) The vitamin G content of lean pork and beef is nearly the same. The average units per gram of dried material for pork are: Raw 6.9, cooked 6.5, and autoclaved 7.3. For dried beef the units are: Raw 4.9, cooked 4.4, and autoclaved 5.1. The data indicate no destruction of vitamin G as a result of cooking or autoclaving.

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AUTOMATICALLY OPERATED SAND-CULTURE EQUIPMENT [1]

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INTRODUCTION

There are described in this paper the principles of an automatically operated sand-culture equipment. This equipment is designed to provide (1) the advantages of large-vessel, or flowing-type, water cultures with regard to the maintenance of solution concentration, and (2) the numerous advantages of sand cultures, such as aeration, iron supply, seedling germination, root environment, and elimination of plant supports.

Students of plant nutrition have relied for a good many years on the use of artificially prepared solutions for the development of information on the essential character and toxicity of the salt constituents of the soil solution. In investigations of purely qualitative character, specialized equipment has been largely uncalled for, but in many directions inquiries of this type have now progressed into quantitative fields; accordingly, it has become increasingly important that the methods employed be such that the concentrations of ions presented to plant roots can be maintained within closely fixed limits.

Briefly stated, with the equipment here described the solution is applied by automatically controlled pumps to the surface of free-draining sand cultures at hourly or other selected intervals. The displaced solution returns by gravity to the supply reservoir. Since the capacity of this reservoir is large as compared with the volume of the solution held by the sand, the maintenance of solution concentrations within reasonable limits resolves itself into a matter of periodic replenishment of the water and salt constituents that are removed by the plants. The frequent automatic replacement of the solution held by the sand prevents any noteworthy changes in the concentrations of elements presented to the plant roots. New solutions are recurrently substituted for those in use. Iron is supplied in the form of water-insoluble minerals mixed with the sand. The present paper gives a detailed description of the construction and operation of this widely adaptable sand-culture principle.

DESCRIPTION

GENERAL

A unit of sand-culture equipment comprises a sand culture, a solution reservoir, and a motor pump. Whether small, as for single plants or seedlings in the greenhouse, or large, as for trees carried into the fruiting stage, the sand cultures are always free-draining. The capacity of the solution reservoir should be large as compared with the daily transpiration demands of the plants. The reservoir is set at a level below that of the sand culture. The solution, intermittently

[1] Received for publication Feb. 14, 1936; issued October 1936.

pumped from this reservoir, is distributed over the top of the sand by suitably arranged perforated pipes, the rate of application being such that a sheet of free water is quickly developed. The volume per application, and therefore the duration of the run, is such that the solution residual from the previous application is displaced either wholly or partly, depending on the volume of the sand and the size of the plants. Drainage returns to the solution reservoir. The equipment is fully automatic. A time clock, with simple adjustments for frequency and duration of flooding, controls a magnetic switch that starts the pumps.

SAND BEDS AND RESERVOIRS

The method for maintaining solution concentrations is applicable to sand cultures of varied characteristics. The type or size of culture vessel depends on the particular experimental purpose served.

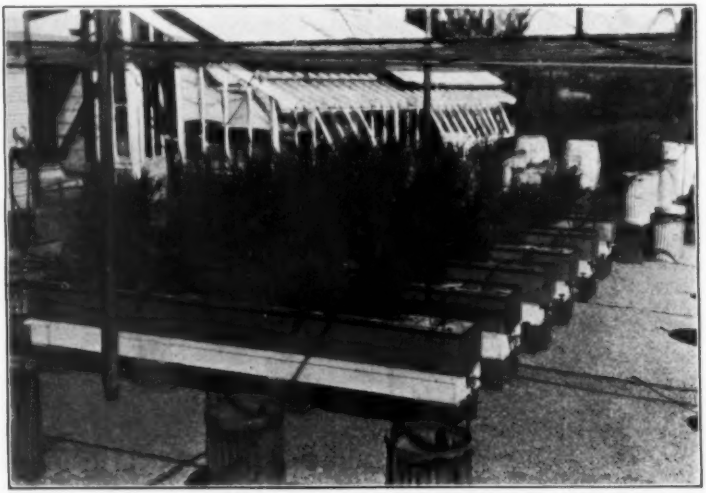

FIGURE 1.—Sand beds of galvanized iron. In each bed there are duplicate plantings of each of four crops in an experiment designed to determine the effect of high and low nitrate concentrations on the tolerance of each of these four kinds of plants to chloride and sulphate salts.

The sand beds described in an earlier publication,[2] and here illustrated as figure 1, have now been equipped with motors, and larger solution reservoirs have been installed. These sand beds, six in number, have surface dimensions of 18 inches by 12 feet. They are 11 inches deep at the sides and 13 inches deep along the center line. The bottom 2 inches of the beds (from the center line) is filled with pea gravel, and above this is placed a ¾-inch layer of coarse sand. The beds are then filled to within 1½ inches of the top with quartz sand. A ¼-inch-mesh screen separates the quartz sand from the coarse drainage sand so that the latter will not be disturbed when the roots of plants are pulled out in harvesting. Beneath the tanks, along the center line, there is riveted and soldered a 2-inch drain roll.

[2] EATON, F. M. A LARGE SAND CULTURE APPARATUS. Soil Sci. 31: 235–241, illus. 1930.

Multiple perforations in the bottom of the sand bed permit drainage into this roll, which in turn drains through a 1-inch pipe to the supply reservoir. A 2-inch overflow pipe connected to the drain roll opens about 1 inch above the surface of the sand. Evaporation and the

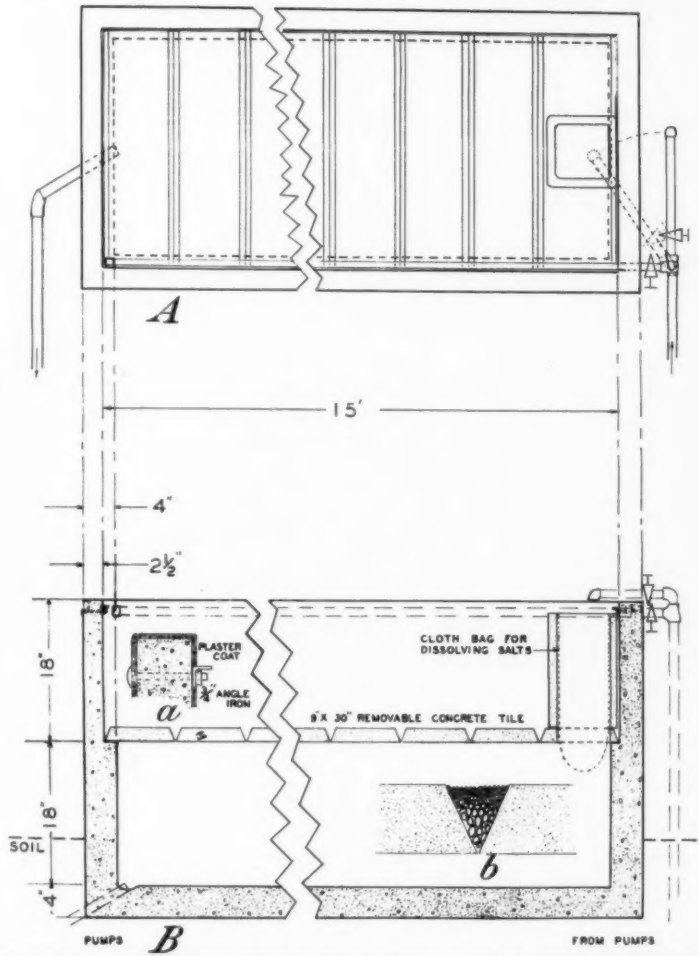

FIGURE 2.—Sections of shallow concrete sand beds to be used for annual crops: *A*, Horizontal section; *B*, vertical section. *a*, Reinforcing angle iron (welded at four corners), which supports perforated metal plates above sand, to suppress evaporation and the growth of algae; *b*, a section at edges of two cross tiles, showing the sand-and-gravel drainage way.

growth of algae are restricted in these beds by perforated galvanized plates supported a little above the surface of the sand. These plates, 12 by 18 inches, are used in pairs that overlap between the rows of plants, the edges next to the plants being turned up about one-half inch to prevent injury to the plant stems.

The sand cultures just described are to be replaced with new equipment of larger dimensions having certain advantages in design. The new cultures (fig. 2), are to be constructed of concrete, with each sand bed and its reservoir poured as a combined unit. The inside surface of the sand beds will be 30 inches wide and 15 feet long, and the depth of the sand will be 13.5 inches. The sand is to be supported above the solution by concrete tile resting on ledges. Each of the reservoirs is to have a capacity of 1,469 liters. The sand, extending to within 2½ inches of the top of the bed, will have a volume of 42.5 cubic feet, or 1, 204 cubic decimeters. Drainage from the sand to the reservoirs is effected through coarse sand and gravel placed between the beveled edges of the supporting tile. An earthen tile 7 inches square that extends from above the sand to an opening made in one of the end supporting tiles provides access to the reservoirs for measuring and renewing the culture solutions. Eight of these culture beds are to be constructed. The solution pumps, operated by belts from a shaft driven by a single motor, will be

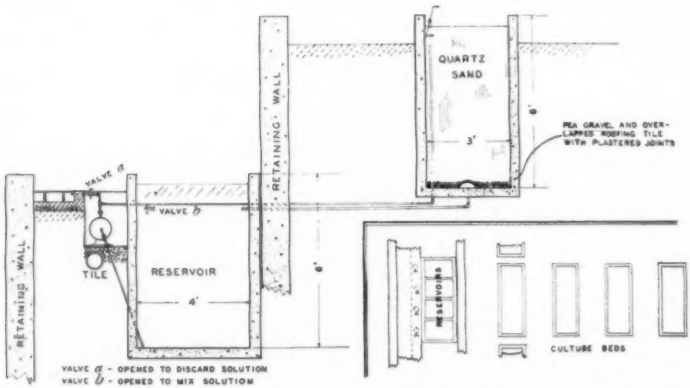

FIGURE 3.—Cross section of one of the deep sand beds with its supply reservoir. The insert shows the arrangement of four of these beds.

assembled in a suitable housing at one end of the experimental enclosure. The pumps, connected by 1¼-inch pipe with the reservoirs and sand-bed outlets, will have discharges of about 50 liters per minute. It is estimated that this complete equipment, exclusive of the culture sand, will cost about $1,000.

The culture vessels used at the Rubidoux Laboratory, Riverside, Calif., for trees (fig. 3) are constructed of concrete, the walls being 4 inches thick, the inside surface 3 by 10 feet, and the depth 6 feet. These beds, 20 in number, rise 1 foot above the ground and extend 5 feet into the ground. The supply reservoirs rise slightly above the surface of the ground on a lower terrace. A 1-inch layer of pea gravel, placed over the surface of permanent cultures of this type to check evaporation and algae growth, takes the place of the galvanized plates used on the metal sand beds where annual plants are grown.

Four sets of six 80-liter galvanized-metal containers are also in use. Each set of these cans has a common reservoir from which solution is withdrawn and to which the solution returns by free drainage. This

equipment permits of four treatments with six different test crops under each.

It is to be recommended that the supply reservoirs for culture solutions be made large. Both water-retaining capacity of the sand and the expected daily volume loss by transpiration when plants are at full growth should be taken into account in planning for reservoir capacity.

Each of the shallow metal sand beds contains 382 cubic decimeters of sand and retains approximately 160 liters of solution against gravity, and each of the supply reservoirs holds 400 liters of solution, making a total of 560 liters of solution in each system. When plants are at full growth in the control bed, a day's transpiration loss in excess of 60 or 70 liters is regarded as high. If the absorption of salt constituents is neglected, then the concentration of the solution in the system would be increased in the order of 11 percent by 60 liters of transpiration. These supply reservoirs, accordingly, are regarded as being too small.

Per unit of sand surface, the reservoir capacity of the new shallow beds of concrete will be approximately double that of the shallow metal beds.

The capacity of each of the reservoirs of the large cultures for trees (fig. 3) is 1,600 liters. About 500 liters of solution is retained by the sand in each bed. Since the daily transpiration of 4-year-old trees grown in these beds will rarely exceed 100 liters, the daily concentration of the solution in the system will be increased by about 5 percent except possibly on occasional days when the saturation deficit is unusually high.

Rust-resistant galvanized metal will give many years of service if used above ground, particularly when a first coat of red-lead paint is applied inside. If properly protected outside by dressings of lead and asphalt, good underground service can be expected also; but such construction cannot be looked upon as permanent, and slow leakage is difficult to detect. When economy has been necessary, 50-gallon iron oil drums have been used; but these will last only a few years.

In connection with this description of metal beds, the comment may be made that there has been no evidence that plant injury has resulted from the use of red-lead paint applied as a first coat to protect the galvanized-metal surfaces from corrosion. Corn plants have been grown without injury in water cultures when a large mat of excelsior dipped in red-lead paint and dried, was inserted into the mason jars used as culture vessels. Plants were likewise uninjured when as much as 20 parts per million of lead as lead acetate was added to culture solutions. In the plumbing for sand-culture solutions with hydrogen-ion concentrations between pH 6 and 8, bronze and brass valves have been used freely without evidence of plant injury.

As a result of surface tension, the lower several inches of sand in cultures remains almost saturated with water after drainage has ceased; this effect, if regarded as undesirable, can be minimized by any one of a number of expedients.

PUMPS AND MOTORS

Several makes of centrifugal pumps with ½-inch outlets are in use, which will deliver solution at a rate of 20 liters per minute against a 6-foot head, when directly connected with motors of one-sixth horse-

power and 1,450 revolutions per minute. A turbine pump, which has a somewhat higher efficiency, is also successfully employed. The centrifugal pumps used for the operation of the deep cultures illustrated in figure 3 are regarded as especially satisfactory. They have ½-inch outlets, and are connected with an induction-repulsion motor of one-fourth horsepower and 1,450 revolutions per minute. These pumps have a discharge of 20 liters per minute against a 20-foot head, and deliver in the order of 40 liters per minute through plumbing arranged as shown in figure 3. The discharge from each pump is carried through a ¾-inch pipe. None of the pumps in use are self-priming; accordingly, it has been the custom to mount them outside the reservoirs, about 15 inches below the normal level of the solution. In the work at Rubidoux Laboratory, solutions are returned to volume each day, and the loss in any one day is never sufficient to drain the pumps. The plumbing of each unit is so arranged that new culture solutions can be circulated for mixing before being applied to the beds.

Consideration has been given to the necessity for using pumps made of special alloys to prevent or reduce corrosion. The abrasion factor introduced by the sand is largely avoided by using care in establishing the coarse sand and gravel below the fine quartz sand. One manufacturer recommended, as satisfactory from the corrosion standpoint, a chrome-nickel-alloy steel, and pointed out that this metal, referred to as one of the 18–8 metals, would offer good resistance to abrasion, being comparable in this respect to cast steel. One of the centrifugal pumps has a cast-iron impeller and the others have bronze impellers; all have given satisfactory service during the first year of use, without material evidence of corrosion.

A sentinel-type breaker switch is installed adjacent to each motor pump, permitting independent operation and providing protection to the equipment.

The motor pumps, according to their type and the favorableness of the quotation, cost from $20 to $50 each, complete with base.

EQUIPMENT FOR AUTOMATIC OPERATION

The automatic operation of the motor pumps at the desired hours and for the desired durations may be effected in a number of ways. Two systems whereby the operation of the motors was limited by light-sensitive elements to the hours of substantial transpiration have been tried and discarded. Both proved to be subject to failure and required attention. The equipment described herein is now employed and is regarded as satisfactory. There is also described a simpler equipment that the writer believes might be suitable for many experimental purposes.

The equipment in use is comprised of (1) a time switch, (2) an auxiliary duration timer, (3) a small interposed auxiliary contactor, (4) a 75-ampere triple-pole magnetic contactor, and (5) a selector switch to permit operation of equipment automatically from the timer or by hand.

(*1*) *Time switch* (G. E. type T–15 time switch).—This piece has an electric clock with a 24-hour dial. The dial carries a series of movable "on" and "off" riders which actuate a mercury-to-mercury switch. The riders may be set to operate the pump equipment at intervals of

1 hour or longer. The minimum period between an "on" and "off" rider is one-half hour.

(2) *Auxiliary timer* (G. E. type TSA–10 timer).—This piece controls the duration of each operation of the motor pumps. The timing element consists of a small synchronous motor. By means of an electromagnetic clutch, the motor drives a pointer across a scale graduated in minutes. An adjustable arm with contact points is set on the scale for the desired time interval of operation. When the moving arm reaches the adjustable arm the contacts are opened, and shut down the motor pumps. The contacts on the adjustable arm are normally closed and are rated to carry 1 ampere at 115 or 230 volts.

(3) *Interposed auxiliary contactor* (G. E. CR 2810–1265).—The contacts of this piece are normally open. Its use is necessitated by the fact that the main contacts of the auxiliary timer (2) are limited to 1 ampere, whereas the operating coil of the magnetic contactor (4) that controls the pump motors will take an operating-coil inrush current of considerably more than 1 ampere. The operating coil of this auxiliary contactor requires only a fraction of an ampere. The current passing though the contacts of the auxiliary timer (2) energizes the coil of this interposed contactor, closing its contacts and thereby completing the secondary circuit through the coil of the magnetic switch (4).

(4) *Seventy-five-ampere triple-pole magnetic contactor.*—This contactor will carry the load represented by upward to 30 motors of one-sixth horsepower each. Either 110- or 220-volt single-phase motors may be operated from this three-pole magnetic switch by connecting them across the appropriate lines. The 110-volt current is used in the operating coil and in pieces 1, 2, and 3.

(5) *Selector switch* (G. E. Type CR 2960–SY103A).—This switch is wired in with the other devices so that the automatic time equipment is inactive when the switch arm is in the "hand" position; when in the "automatic" position, operation of the pump motors is entirely dependent on the timing-control circuit, and when in the "off" position the motors are shut down and are not affected by the operation of the timing equipment. The piece may be dispensed with by installing a snap switch across the lines from the contacts of the interposed contactor (3) to the magnetic contactor (4). Either arrangement permits one to run the motors independently of the timing device when solutions are to be mixed or the reservoirs emptied.

The external wiring diagram for connecting these pieces is simple and can be readily sketched by a manufacturer's representative. The external sketch, however, has little meaning except in conjunction with a study of the internal wiring diagrams of the several pieces which become somewhat complex and are not easily reproduced.

Briefly stated, one operation carries through as follows: An "on" rider on the time clock (1) closes the mercury-to-mercury switch and a 115-volt line current flows through the normally closed contacts of the auxiliary duration timer (2), the clock motor of which is started, and on through the coil of the normally open interposed auxiliary contactor (3). The contacts of the latter close, completing a second 115-volt circuit through the operating coil of the large 75-ampere magnetic switch (4), which contactor closes, starting the pumps. The pointer of the auxiliary timer (2) moves on by clock and separates its

contacts at the end of the desired duration period. The breaking of this circuit deenergizes the coil of the interposed auxiliary contactor (3), allowing its contacts to open, which in turn breaks the circuit that has held the magnetic contactor (4) closed. The pumps are stopped at this point, but the cycle of the timing mechanism is not yet complete. When an "off" rider on the time switch (1) is reached, its mercury switch is opened. The opening of this switch automatically resets the auxiliary duration timer (2) for the next action.

The above control apparatus can be installed at a cost of about $100.

For some purposes the equipment for controlling pump operations may be greatly simplified by employing a time switch (G. E. type T–27) that has recently become available. As differing from the time switch (1), which has been described, an "off" rider of the newer type may be set to follow an "on" rider as closely as desired, but the minimum interval between "on" riders on the 24-hour dial is 1 hour and 45 minutes. The duration period cannot be adjusted as closely with these riders as it can be with the auxiliary timer described under (2). This timer employs, in place of the mercury-to-mercury switch, silver-button contacts capable of carrying a connected starting load of 40 amperes. A limited number of pump motors, accordingly, could be connected directly to the timer without accessory apparatus. Either with or without the magnetic switch, the time interval between operations can be reduced to 53 minutes by using two of these timers connected in parallel. The two timers, by appropriately setting the "on" riders, would alternate in operating the motor-pump or magnetic-switch circuits. The list price of this timer is about $20.

OPERATION

SELECTION OF SAND

Some importance is to be attached to the selection of quartz sand with particle sizes best suited to culture uses. If the sand is too coarse little water is held against gravity, whereas if the sand is too fine drainage is slow. Particularly undesirable is sand with particle sizes so mixed that little void remains when the sand has settled. Although this matter needs further study, it appears probable that no difficulty would be experienced with any sand held by a 100-mesh screen after passing a 40-mesh screen. A very satisfactory beach sand, taken at the foot of the Torrey Pines grade in southern California, had by weight 2 percent of 20–40-mesh particles, 25 percent of 40–60, 54 percent of 60–80, 13 percent of 80–100, and 6 percent of particles that passed a 100-mesh screen.

IRON SUPPLY

Experience at this laboratory has indicated that 0.1 percent of magnetite mixed with quartz sand makes unnecessary the use of soluble iron in the culture solutions maintained on the acid side of neutrality. A number of crop plants obtain sufficient iron from magnetite when the pH value is as high as 8. Pyrites may also be used as a source of iron if the medium is alkaline; on the acid side of neutrality, pyrites sometimes causes injury.[3] These data support the conclusion that iron may be obtained by plants from some water-

[3] EATON, F. M., BLAIR, G. Y., and WILCOX, L. V. [Unpublished data.]

insoluble minerals by absorption across the particle-root interface, rather than by absorption from the culture or soil solution. The beach sand previously referred to in the present paper contains, in addition to constituents other than quartz, water-insoluble iron that is available to plants. Corn plants, which have a relatively high iron requirement, when grown in this sand showed no evidence of chlorosis if supplied with nutrient solutions maintained at pH values 5 to 6, 8, and 9.

CONTROL OF ORGANISMS

Free-living and pathogenic organisms tend to develop in sand cultures, particularly when the surface of the sand is not protected by gravel or metal plates to repress the growth of algae. Such an effect may become pronounced after several successive crops. It has, accordingly, become the writer's custom to apply a 0.5- to 1-percent solution of formaldehyde between crops. This solution is circulated by the pumps four or five times a day for several days, and is then washed out by several changes of water. The design of the equipment is such that steam or hot water can likewise be used for sterilization.

SALT ACCUMULATION AND DAMPING-OFF

Some minor evidence of accumulation of salts of low solubility on plant stems has been experienced when the concentrations of chlorides and sulphates have been made high in plant-tolerance studies. This has not been observed in the control beds, where only the usual nutrient solutions were used. The fact that solutions are applied at frequent intervals to a depth of 1 inch over the surface of the sand and then quickly drained away is doubtless largely responsible for general absence of salt incrustations. The lower portions of the stems, up which salt might creep from the sand, are submerged by each application of solution. The metal plates and gravel used to check algae growth serve likewise to reduce evaporation rates near the surface of the sand.

In the early seedling stages, when plants are most subject to damping-off, they transpire very little water. During this period root development is relatively rapid and it is not necessary to circulate the culture solutions, with the attendant wetting of the surface of the sand, since sufficient water and nutrients are supplied by the culture solution retained by the sand against drainage.

PRACTICAL SUGGESTIONS

USE OF SAND CULTURES IN HOTHOUSES

In a previous publication [4] the suggestion was offered that sand cultures might have practical advantages over soils in hothouse culture. Sand cultures are now being used commercially to a limited extent. In many cases plants supported by nutrient solutions in sand cultures have developed more rapidly and have been more fruitful than those grown in soils under otherwise similar conditions. Practical considerations incline the writer to the view that the usefulness of sand cultures is in no way limited to nutritional studies. The idea of maintaining the hydrogen-ion concentration and the concentrations and proportions of nutrients at levels best suited to the varied

[4] EATON, F. M. See footnote 2.

requirements of plants grown in hothouses is an inviting one, and there is likewise to be taken into account the ease with which soil organisms may be controlled.

Sand beds of the type shown in figure 2 are regarded as being well-adapted to greenhouse use. If judicious use is made of thermostats, culture temperatures may be closely controlled by installing steam pipes or electrical heating cable in the sand. The temperature of the culture solutions can likewise be controlled.

Plants may be removed from sand cultures for transplanting to the field or pots with a minimum of injury to the root systems.

COMPOSITION OF CULTURE SOLUTIONS

The following material on the composition of nutrient solutions will be wholly familiar to students of plant nutrition. It is offered for the convenience of those whose interests are primarily in the propagation and culture of plants.

Plants will make a creditable growth when supplied with culture solutions of markedly different composition. A highly satisfactory culture solution for some kinds of plants may be poorly suited to others, and a solution that is superior during one season of the year may not be the most desirable in another season, since climatic conditions, as well as length of day, influence the nutrition of plants. During recent years there has been a tendency toward the use of solutions that are less concentrated than many of those employed by the earlier investigators.

The composition of two culture solutions is shown in table 1. The first of these is a satisfactory and extensively used nutrient popularly known as Hoagland's solution. The second solution, is one that has been used effectively in nutritional studies in sand cultures at the Rubidoux Laboratory.

TABLE 1.—*Composition of 2 nutrient solutions*

Solution and constituents	Millimoles per liter	Grams per 100 liters
Hoagland's solution:		
Calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$	5	[1] 118 (91)
Potassium nitrate, KNO_3	5	51
Magnesium sulphate, $MgSO_4 \cdot 7H_2O$	2	49
Potassium acid phosphate, KH_2PO_4	1	14
A Rubidoux Laboratory solution:		
Calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$	4	[1] 94 (72)
Potassium nitrate, KNO_3	3	30
Ammonium sulphate $(NH_4)_2SO_4$	2	27
Magnesium sulphate, $MgSO_4 \cdot 7H_2O$	2	49
Potassium acid phosphate, KH_2PO_4	.2	3

[1] Weights in parentheses are for granular calcium nitrate with 15.5 percent nitrogen.

Iron must be made available to plants supported by nutrient solutions. Mention has been made of the suitability of certain water-insoluble iron minerals. Iron citrate can be added to culture solutions at the rate of 1 cc of a 0.5-percent solution per liter of nutrient each several days or sufficiently often to maintain the plants in good condition. Like amounts of specially prepared iron humate,[5] iron tartrate, or ferrous sulphate are also effective.

[5] HORNER, C. K., BURK, D., and HOOVER, S. R. PREPARATION OF HUMATE IRON AND OTHER HUMATE METALS. Plant Physiol. 9: 663-669. 1934.

In addition to iron and the elements of the base nutrient solution, plants require small amounts of certain other elements. Boron, manganese, and zinc should be introduced. For most purposes 1 p. p. m. of boron, 0.2 p. p. m. of manganese, and 0.1 p. p. m. of zinc will prove satisfactory. To approximate these concentrations in 100 liters of solution, add 0.6 g of boric acid (H_3BO_3), 0.1 g of manganese chloride ($MnCl_2.4H_2O$), and 0.04 g of zinc sulphate ($ZnSO_4.7H_2O$). It is not implied that other elements in small quantities are not beneficial, but the need of particular ones in addition to the amounts introduced as impurities with the principal salts has not yet been clearly defined. Many elements are contained in quartz sand, and they are likewise introduced if tap water is used in the preparation of solutions. So-called A-to-Z mixtures of miscellaneous elements, in very small amounts, are sometimes added to culture solutions, as are extracts of manure and soils.

The phosphate in culture solutions tends to precipitate the iron. Recent work by Olsen[6] shows further that absorbed iron may be precipitated and rendered inactive within the plant when the phosphate content of the nutrient is high. Hoagland's solution is often made up with only a tenth as much potassium phosphate as that given in the formula, and the phosphate content of the second solution is purposely made low for this reason.

For general-purpose culture solutions it is desirable to introduce a part of the nitrogen as ammonium ion. Unless tap water is used in making up the nutrient, 2 millimoles per liter of sodium chloride, NaCl (12 grams per 100 liters), should be added. The growth of some plants, tomatoes for example, will be substantially improved as a result of introducing this salt.

Tiedjens,[7] working with apples and tomatoes, has reported that nitrate ion was assimilated most satisfactorily when absorbed from an acid solution of approximately pH 4.0 whereas ammonium ion was assimilated most satisfactorily when absorbed from nutrient solutions having pH values of 5.0 to 6.5, varying somewhat with different varieties. In general, ammonium and nitrate salts produced equally good growth provided their limitations were recognized. Trelease and Trelease,[8] have proposed the use of solutions balanced with respect to NO_3/NH_4 ions to maintain stable hydrogen-ion concentrations.

If culture solutions are extensively employed, consideration can properly be given to the use of technical and commercial grades of chemicals, but some caution must be employed in their selection. The synthetic commercial fertilizers, such as ammonium sulphate and granular calcium nitrate, are usually relatively pure.

The frequency with which old solutions must be discarded and new ones substituted is always an individual problem, related, of course, to the volume of the supply and the size of the plants. Potassium, ammonium, and nitrate ions tend to become deficient in culture solutions more rapidly than some others. These and other constituents can be replaced as they are taken up by plants if laboratory facilities for their quantitative determination are available; otherwise it is desirable to substitute new solutions at frequent intervals.

[6] OLSEN, C. IRON ABSORPTION AND CHLOROSIS IN GREEN PLANTS. Compt. Rend. Lab. Carlsberg, Sér. Chim. 21 (3): 15–52, illus. 1935.

[7] TIEDJENS, V. A. FACTORS AFFECTING ASSIMILATION OF AMMONIUM AND NITRATE ION, PARTICULARLY IN TOMATO AND APPLE. Plant Physiol. 9: 31–57, illus. 1934.

[8] TRELEASE, S. F., and TRELEASE, H. M. PHYSIOLOGICALLY BALANCED CULTURE SOLUTIONS WITH STABLE HYDROGEN-ION CONCENTRATION. Science (n. s.) 78: 438–439. 1933.

DISCUSSION

The electrical and mechanical features of the sand cultures here described have required little attention other than an occasional greasing and oiling of the pumps and motors. The unattended operation of the equipment on Saturday afternoons and Sundays has been entirely satisfactory. If much phosphate is used in culture solutions, it appears possible that calcium phosphate might accumulate in the pumps to such an extent that they would require cleaning.

The best quartz sand contains impurities that are not removed by digestion in strong acid, and for that reason water-culture technique is essential to some research. In water-culture technique practically all of the advantages are in favor of the large-vessel aerated type. Many workers have found flowing cultures inadequate as a means of maintaining uniform concentrations, except at the expense of wasting large volumes of solution, because the diverse conditions that exist between day and night and at the successive stages of plant development defy corresponding adjustments in the rate of supply of new solution. Problems concerned with seedling germination, aeration, iron supply, plant supports, and with less tangible matters attend the use of all forms of water culture. These difficulties are minimized in sand cultures, the use of which limits the care that must be devoted directly to plants during their growth to such operations as thinning, spraying for pests, and staking to prevent wind injury. The labor-saving advantages, however, may be regarded as somewhat incidental to the opportunity afforded, by the equipment here described, for closely controlling and defining the concentrations of solution constituents in sand cultures. In the majority of investigations, the traces of miscellaneous elements introduced by the quartz sand may be regarded as advantageous.

SUMMARY

The improved automatically operated sand-culture equipment described herein is designed to provide (1) the advantages of large-vessel, or flowing-type, water cultures with regard to the maintenance of solution concentration and (2) the numerous advantages of sand cultures, such as aeration, iron supply, seedling germination, root environment, and elimination of plant supports.

The solution is applied by motor pumps controlled by a time clock to the surface of free-draining sand cultures at hourly or other selected intervals. The displaced solution returns by gravity to the supply reservoir.

Equipment in use at the Rubidoux Laboratory is described, and suggestions are offered on the construction of sand beds and solution reservoirs, on types of sand, iron supply from water-insoluble minerals, control of troublesome organisms in the sand, and the applicability of the method to hothouse culture.

A brief account of the composition of certain culture solutions is given for the convenience of those interested in the propagation and culture of plants.

THE DEVELOPMENT OF THE WHEAT SPIKE[1]

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INTRODUCTION

All parts of a wheat stem arise from its growing point. In the early stages of growth only leaves are produced, while in later stages the spike and its parts are produced. The time of the transition from the early, or vegetative stage, to the later, or reproductive stage, of development has a bearing upon the earliness and lateness of heading of wheat varieties. The effect of low-temperature germination (vernalization) in bringing about earlier heading in certain varieties of wheat, is related to the effect of such treatment upon earliness of spike differentiation.

Some studies of the development of the wheat spike have been published, but more attention has been given to the development of the kernel than to that of the spike. An early contribution to a better understanding of the development of the wheat plant was made by Carruthers (*2*).[2] Jensen (*4*) has cited a number of publications dealing with certain phases of morphological development of the wheat plant, and he has described and illustrated various phases of spike and flower development. More recently Percival (*8*), Kiesselbach and Sprague (*5*), and Noguchi (*7*) have described and presented line drawings of certain phases of spike and spikelet development. McCall (*6*) has studied the developmental anatomy and morphology of the embryo and seedling of wheat.

This is the second of a series of articles dealing with the ontogeny of the inflorescence of small grains. The first article dealt with the development of the barley spike (*1*). The present article describes briefly, and illustrates with photomicrographs, the principal stages in the development of the growing point, the spike, and the spikelet of the wheat (*Triticum aestivum* L.) plant.

MATERIALS AND METHODS

The methods used in handling the dissected material and in taking most of the photomicrographs were the same as those described in the earlier paper on barley (*1*). Some of the photographs were taken with a 32-mm Micro Tessar lens.

The plants from which the growing points and spikes were dissected were grown in the field. Collections were made from time to time during the growing season. Most of the plants used were Purkof, a beardless, semihard, red winter variety of wheat.

DESCRIPTION OF SPIKE DEVELOPMENT

The growing point of a head-bearing wheat stem in its growth from germination to pollination passes through two stages of development.

[1] Received for publication Feb. 24, 1936; issued October, 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 450.

The first is the vegetative stage, which extends from seeding in the fall through the winter to late March or early April. In this stage the internodes of the stem remain short and only leaf initials are produced by the growing point. The growing point elongates but remains nearly smooth in outline. This is also the stage of active tiller development. The beginning of the second, or reproductive stage, is indicated by the appearance of double ridges, the upper member of which develops into the spikelet initial. During the reproductive stage certain of the internodes of the stem elongate and the spikelet parts differentiate and increase in size. The vegetative stage is shown in plate 1, *A*, *B*, and *C*, and the reproductive stage in plate 1, *D*, to plate 2, *F*, inclusive. Thus, it is seen that the developmental stages of the wheat plant are very similar to those of barley, already described (*1*).

The shoot of the embryo of a wheat kernel has only a few parts. They are the coleoptile, the first, second, third, and fourth leaf initials, the growing point, and a tiller bud in the axil of the coleoptile.

The parts of the shoot and their arrangement in the embryo can be observed in seeds which have been soaked for about 24 hours. The coleoptile completely encloses the leaf initials and the growing point. In the axil of the coleoptile, opposite the scutellum, the tiller bud with its first (prophyllum) and second leaf initials, and the growing point can be located. The first leaf if slightly shorter than the coleoptile, which also encloses the growing point, and its fold is on the side next to the scutellum. The second and third leaf initials only partly enclose the growing point. The fold of the second leaf initial is on the side opposite the scutellum, and the fold of the third leaf is on the same side as the first leaf. Across the growing point on the side next to the scutellum is a ridge, which is the beginning of the fourth leaf. A more detailed description of all the structures mentioned has been given by Percival (*6*).

By the time a wheat plant has two leaves the sixth leaf initial can be easily seen (pl. 1, *A*, l_6). The rest of the leaves range in size from the fifth leaf initial, which nearly encloses the growing point, up to the fully matured first leaf. The growing point at this time is short and hemispherical (pl. 1, *A*, *g*).

Leaves begin their development as lateral, alternate ridges (pl. 1, *B*, *x*) beneath the apex of the growing point. The leaf initials arise in acropetal order, become larger on passing farther from the tip, and nearly encircle the growing point. Each ridge is most prominent on the side opposite the leaf initial just beneath it. The more prominent side of the ridge forms the apex of the young leaf (pl. 1, *A*, l_5, and pl. 2, *C*, *l*), and this is the oldest portion of the leaf since grass leaves elongate by basal growth.

Each leaf in succession grows up over the growing point or the spike and inside of the preceding leaf. A leaf partly enclosing the spike is shown in plate 2, *C*. This leaf was removed and a photomicrograph was taken to show the spike and the size of the succeeding leaves (pl. 2, *D*, *l* and *l'*). The next leaf (pl. 2, *D*, *l*) has just started to elongate. The two leaf initials appear to be the last initials formed on this stem.

The vegetative stage of stem development in winter wheat is shown clearly in plate 1, *B*. This growing point was removed on October 17 from one of the largest stems of a volunteer wheat plant 50 to 60 days

old. The growing point is short. Ten leaves were removed to expose the growing point and there still remained four well-developed leaf initials at the base and four ridgelike leaf initials farther up on the growing point.

When active growth starts in the spring the growing point begins to elongate in preparation for spike differentiation (pl. 1, *C*, *g*). Leaf initials can be seen at the base of the growing point and there is some indication of ridges higher up, but in contrast with the growing point in plate 1, *B*, *g* it is relatively smooth in outline. When this plant was sampled on March 28, the internodes of the stem had not begun to elongate.

After the elongation of the growing point the first indication of spikelet development is the appearance of double ridges (pl. 1, *D*, *x*, and *E*, *x*). The lower or smaller ridge of the pair can be seen until the upper member of the pair becomes large enough to cover it. The upper ridge elongates and attains considerable size and prominence before the spikelet structures begin to differentiate (pl. 1, *F*, *si*).

It is of interest to note the changes in the shape of the spike and the difference in the size of the spikelet initials in either direction from the middle (pl. 1, *F*). The shape of the spike at this stage is lanceolate. The spikelet initials show the greatest elongation in the middle of the spike (pl. 1, *F*, *si*). From the middle toward the base and tip of the spike, the spikelet initials are successively shorter, but the tip spikelets are not as long as those at the base.

Differentiation of each of the spikelet structures begins first in those middle spikelets which show the greatest elongation (pl. 1, *F*, *si*), and in succession in other spikelets of the head basipetally and acropetally. The terminal spikelet (pl. 1, *F*, *t*) is the last to differentiate. This sequence of development among the spikelets is maintained throughout the development of the spike, including anthesis and kernel development. In the mature spike the order of the average kernel weight of a spikelet is the same as the order of spikelet differentiation, viz, the heaviest kernels are in the spikelets in the middle of the spike where differentiation first begins, and they decrease in weight from this point toward the base and the tip.

The empty glumes are the first spikelet structures to differentiate (pl. 1, *G*, *c*). They appear as transverse ridges on both sides of the spikelet initial. A little later other ridges appear just above and parallel to the first. The second ridges become the lemmas of the first and second flowers (pl. 1, *H*, *gl*). Flower parts develop from the hemispherically shaped meristem above the lemma initial (pl. 1, *H*, *r*).

Anther differentiation begins in the basal flowers of the spikelets located about halfway up the spike. Three slight elevations appear upon the meristem above the lemma initial and these soon enlarge (pl. 1, *I*, *an*).

A wheat spikelet is many-flowered. This is shown in plate 2, *A*, fl_1 to fl_5 and *E*. The first-mentioned figure represents a spike of a bearded wheat, sampled May 2, and chosen to give an idea of the appearance of the spike and the arrangement of the flowers in the spikelets. Flower position in the spikelet is better shown by the ventral (left) and dorsal (right) view of the two wheat spikelets in plate 2, *E*. Each spikelet has six flowers arranged alternately and attached to the short rachilla which is hidden by the flowers. Flowers

1 to 3, inclusive, show anthers. Anther differentiation has just begun in flower 4, but none can be seen in flowers 5 and 6. The beginning of awn development can be seen in plate 2, *E*, *a*.

As mentioned above, flower differentiation and development in the wheat spikelet proceeds from the base upward in acropetal order. The empty glumes on both sides of the base of the spikelet differentiate first. Soon the lemmas of the first and second flowers appear, and the meristem from which the stamen and pistil differentiate becomes prominent and hemispherical. Differentiation in the third, fourth, fifth, and sixth flowers follows in the order named. As shown in plate 2, *E*, the stages of flower development range from those at the base with all parts fully differentiated to those at the tip with the lemma just differentiating.

By the time the last internode (peduncle) of the stem begins to elongate the glumes have enclosed the anthers and pistil (pl. 2, *B*). The awns of the awned variety have considerable length and show the presence of barbs.

It should be noted in plate 1, *H*, *t*, and *I*, *t*, that the wheat spike terminates in an apical spikelet, and hence may be classed as a determinate inflorescence. Before the spikelet and flower parts begin to differentiate (pl. 1, *F*) the number of spikelets that a wheat spike will have, has already been determined.

Only one stage of pistil development is shown (pl. 2, *F*). A portion of the flower has been removed to show the pistil. At this stage the ovary and styles are well formed but the stigmatic branches have not yet appeared.

The plants in plate 1, whether or not grown the same year, show the various stages in the development of the growing point and spike as then would be found at the approximate time of year indicated, under the climatic conditions prevailing at Urbana, Ill. The plant shown in plate 1, *A*, was sampled on October 19, 1935, and that in plate 1, *B*, on October 17, 1935. Plants shown in plate 1, *C* and *D*, were sampled on March 28 and April 2, 1935, respectively. The dates of sampling the other plants shown in plate 1 were as follows: *E*, April 10; *F*, April 21; *G*, April 28; *H*, April 28; and *I*, May 5 in 1934.

DISCUSSION

The probable morphological significance of the lower of the pair of ridges, which mark the transition of the growing point of wheat and barley (*1*) from the vegetative to the reproductive phase, should be pointed out. It is clear from observation that all of the spikelet

EXPLANATORY LEGEND FOR PLATE 1

A.—Leaf initials and growing point of a two-leaved plant of wheat: l_6, Sixth leaf initial; l_5, fifth leaf initial; *g*, growing point. ×35.

B.—Growing point from a large stem of a volunteer plant of winter wheat: l_{11}, Eleventh leaf initial; *x*, first stage of a leaf initial; *g*, growing point. ×30.

C.—Elongated growing point just before spikelet differentiation: *l*, Leaf initial; *g*, growing point. ×30.

D.—Beginning of spikelet differentiation shown by double ridges on a young spike of wheat: *x*, Upper of a pair of ridges. ×20.

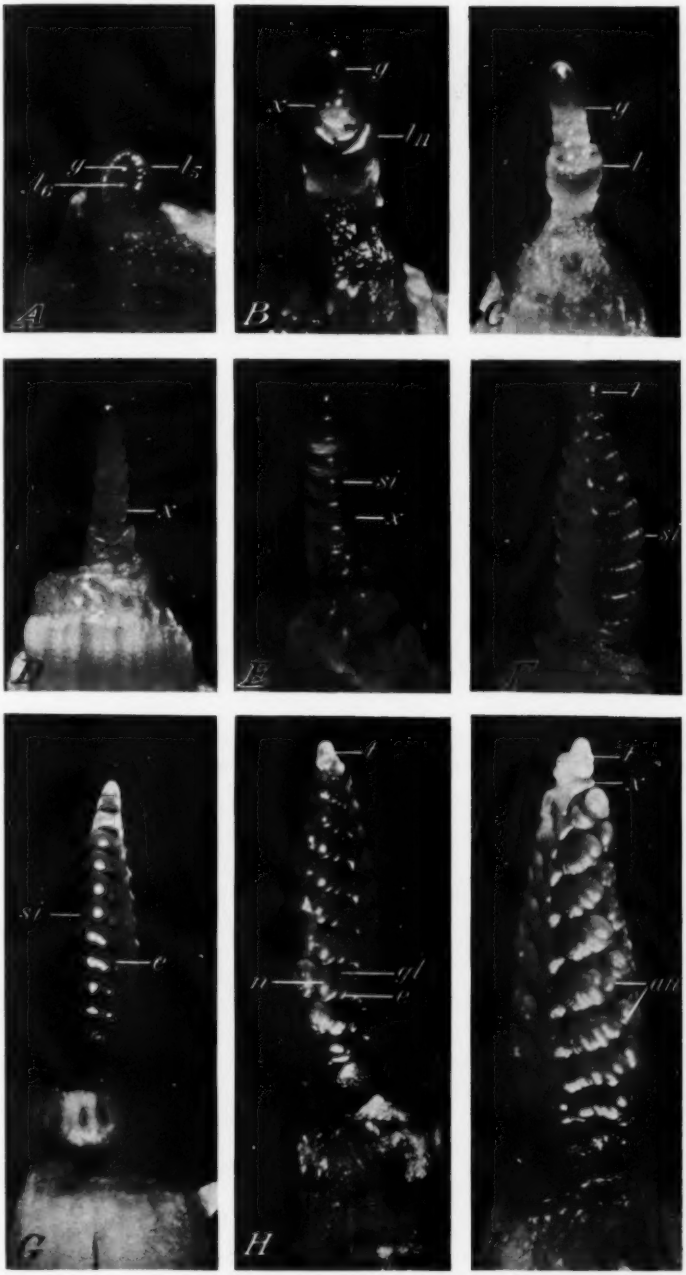
E.—Early stage of spikelet formation in a spike of wheat: *x*, Lower of a pair of ridges; *si*, spikelet initial ×25.

F.—Spikelet development on a spike of wheat just before the beginning of the differentiation of the spikelet parts: *si*, Spikelet initial; *t*, terminal spikelet initial. ×25.

G.—The beginning of glume differentiation on a spike of wheat: *e*, Empty glumes; *si*, spikelet initial. ×20.

H.—A spike of wheat showing well-differentiated glumes: *e*, Empty glume; *gl*, lemma; n, meristem from which anthers and pistil form; *t*, terminal spikelet initial. ×20.

I.—First stage of anther differentiation on a spike of wheat: *an*, Anther initials; *x*, empty glume initial of terminal spikelet; *t*, terminal spikelet initial. ×20.



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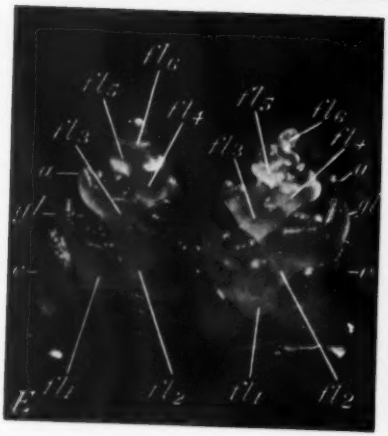

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parts differentiate from the upper of the pair, but the lower ridge is soon covered by the growth of the spikelet, and hence, its history can only be inferred. An explanation suggested by the writer for barley was that the lower of the pair becomes the internode of the rachis. This explanation was not correct, although, as will be pointed out later, the ridge does merge with the tissues of the node.

In a study of the influence of temperature during germination on the subsequent development of certain cereals, Purvis (*9*) noted double ridges on the growing point. She pointed out that the double ridges suggested a leaf and its axillary bud. This suggestion is accepted as correct. Hitchcock (*3*) states: "The spikelet is, therefore, a reduced modified shoot in which the rachilla is a stem bearing at each node a reduced leaf (bract)." Since lateral shoots of grasses originate from the main shoot in the axil of the leaf sheath, the view that the upper ridge from which the spikelet differentiates is an axillary bud, and the lower ridge the rudiment of a leaf, is in harmony with the morphology. The mature spike of wheat as well as of barley has a rudimentary leaf and a spikelet in its axil at the first node and a distinct ridge at the next higher node. At the other nodes of the mature spike the ridge cannot be seen but merges with the nodal tissue.

Since a plant cannot anticipate its environment it must be able to make adjustments to growing conditions. Wheat and barley (*1*) spikes present interesting contrasts in their manner of making adjustments to the environment. Two-rowed barley has only one, and six-rowed barley has only three, single-flowered spikelets that may become fertile, at each joint of the rachis. However, the barley spike is an indeterminate inflorescence and any variation in the number of flowers in a spike can take place at the tip of the spike. The wheat spike, on the other hand, is a determinate inflorescence which terminates in an apical spikelet, so that as soon as the spikelets differentiate their number is fixed. While only one spikelet develops at each node of the rachis, each wheat spikelet has six or more flowers. In most varieties of wheat the two basal flowers of each spikelet are fertile, but under good growing conditions more flowers may become fertile. Thus in barley any variation in the number of flowers in a spike takes place at the tip of the spike, while in wheat any variation in the number of flowers in a spike takes place within the spikelets.

If good conditions for growth, are followed by poor conditions adjustments take place in an orderly sequence. On the spike the last-formed spikelets are the first to cease development and become abortive. Thus abortive spikelets in both wheat and barley are normally found at the base and tip of the spike. Likewise in multiflowered spikelets the last-formed, youngest flowers are the first to suffer under adverse growing conditions.

EXPLANATORY LEGEND FOR PLATE 2

A.—Young spike of wheat showing development and arrangement of flowers in the spikelets: fl_1 to fl_5, Basal, or first flower, to fifth flower, respectively; *an*, anthers. ×30.

B.—Spikes of a bearded and a beardless variety of wheat, at the beginning of the elongation of the peduncle. ×2.5.

C.—A spike of wheat showing how leaves grow up over the spike: *l*, Leaf initial; *s*, spike. ×44.

D.—Same spike as in plate 2, *C*, but with large leaf removed to show size of next smaller leaves and the stage of spike development: *l* and *l'*, Leaf initials. ×44.

E.—Ventral (left) and dorsal (right) views of dissected spikelets of wheat: *e*, Empty glumes; *gl*, lemma; *a*, awns; fl_1 to fl_6, flowers. ×20.

F.—A flower with a portion removed to show a stage of pistil development: *p*, Pistil; *an*, anthers. ×20

SUMMARY

A study of the morphological development of the growing point and spike of wheat was made by dissecting the growing points and spikes from stems at different stages of development. Photomicrographs of the principal stages are presented.

In the resting stage of the seed the stem portion of the wheat embryo consists of the following parts: Coleoptile, first, second, third, and fourth leaf initials, the coleoptile tiller bud, and the growing point. Each of the parts mentioned can be dissected from a seed that has been soaked in water for a few hours.

Leaf initials appear as alternate ridges which nearly encircle the growing point. The ridges are more prominent on the side opposite the leaf initial just below or above it. The prominent portion forms the apex of the young leaf. Leaves elongate by basal growth and grow up inside the preceding leaf.

During the fall, winter, and early spring the growing point remains in the vegetative stage. In the fall the growing point produces only leaf initials, while in early spring, in addition to the production of leaf initials, the growing point elongates. The beginning of the reproductive stage is marked by the apperance of double ridges the upper of which produces the spikelet and its parts. During the reproductive stage the spikelets and spikelet parts differentiate and increase in size.

Spikelet differentiation begins in the middle of the spike and proceeds toward the base and the tip of the spike. Likewise, each of the spikelet parts in the sequence of spikelet development appears first in the spikelets in the middle of the spike.

Within the spikelet, differentiation begins at the base of the spikelet and proceeds upward. Within the flower, the sequence of differentiation of its members proceeds from the outside inward. The pistil and its parts are the last to differentiate.

Spikelet parts differentiate in the following order: Empty glumes, and flowers 1, 2, 3, etc. Within the flower the order of differentiation is: Lemma, anthers, palea, and pistil. The sequence of the pistil parts is: Ovary, styles, and stigmas.

The wheat spike is a determinate inflorescence. It terminates in an apical spikelet placed at a right angle to the plane of the rest of the spikelets. When the spikelets differentiate the number is fixed but adjustment to growth conditions may be made in the number of fertile flowers in a spikelet.

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EFFECT OF PACKING-HOUSE BYPRODUCTS, IN THE DIET OF CHICKENS, ON THE PRODUCTION AND HATCHABILITY OF EGGS [1]

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INTRODUCTION

In an earlier paper Byerly, Titus, and Ellis [2] stated that some lots of meat meal are deficient in some substance that is necessary for the production of eggs capable of supporting embryonic life during the second week of incubation.

In view of the importance of the byproducts of the packing house as protein supplements in the feeding of poultry, it was decided to study further the effect of these materials on the production and hatchability of eggs. This paper presents the pertinent data on the subject obtained by the writers from the fall of 1929 to the fall of 1934, at the Animal Husbandry Experiment Farm of the National Agricultural Research Center, Beltsville, Md.

EXPERIMENTAL METHODS AND MATERIAL

The experiments involved feeding tests with Single-Comb Rhode Island Red pullets. The duration of each experiment was twelve 4-week periods, or 336 days. During each 4-week period some or all of the eggs laid were incubated. The pullets were distributed among the pens so that each contained from 15 to 40 birds. They were selected and distributed in a manner to insure uniform lots with respect to size, weight, and age. These pens received diets containing various protein supplements to be appraised experimentally. The birds were comfortably housed; straw litter was used; feeding was conducted in a uniform manner; and accurate egg records were insured by trap-nesting. Further details regarding selection of the birds, their housing, feeding, and management are given in a paper by Byerly, Titus, and Ellis.[3]

The present experiments dealt chiefly with the effects of packing-house byproducts on production and hatchability of eggs. Data also were obtained on the influence of the various diets on live weight, weight of eggs, feed consumption, and embryonic mortality. The study involved several different methods of approach. One of these, that of the first year (1929–30), was a comparison of a typical packing-house byproduct, desiccated meat meal, with (1) a diet containing no protein supplement and (2) a diet containing a meat-fish-milk supplement, already known to be excellent in its effect on egg production and hatchability. The diet containing the meat-fish-milk

[1] Received for publication Dec. 12, 1935; issued October, 1936.

[2] BYERLY, T. C.; TITUS, H. W.; and ELLIS, N. R. EFFECT OF DIET ON EGG COMPOSITION. II. MORTALITY OF EMBRYOS IN EGGS FROM HENS ON DIETS CONTAINING PROTEIN SUPPLEMENTS OF DIFFERENT ORIGIN. Jour. Nutrition 6: 225–242, illus. 1933.

[3] BYERLY, T. C., TITUS, H. W., and ELLIS, N. R. PRODUCTION AND HATCHABILITY OF EGGS AS AFFECTED BY DIFFERENT KINDS AND QUANTITIES OF PROTEINS IN THE DIET OF LAYING HENS. Jour. Agr. Research 46: 1–22, illus. 1933.

supplement, which is not a packing-house product, may be considered as a positive control and the diet containing no protein supplement as a negative control. Further comparisons of the positive and negative control diets were made the 2 following years. During the fourth year the experiment dealt with comparisons, for poultry feeding, of other packing-house byproducts as prepared by ordinary commercial methods. During the fifth year (1933–34) the test dealt principally with all-beef scrap prepared by methods involving close control over periods and temperatures of cooking.

Throughout the entire period of the experiments the diets contained an adequate supply of necessary mineral elements. In the first year's work a fixed quantity of mineral mixture was incorporated in each diet, whereas during the last 4 years all diets contained a fixed quantity of anhydrous sodium sulphate and common salt (0.5 percent of each) but different quantities of ground limestone and steamed bonemeal. In 1930–31, the quantities of the last two materials were so adjusted as to keep the phosphorus content between 0.5 and 0.9 percent and have a calcium-phosphorus ratio of 4:1. In each of the ast 3 years, 1931–34, the adjustment was so made that all the diets contained approximately 1.2 percent of total phosphorus and 3.0 percent of total calcium.

BASAL DIETS

In compounding each of the diets studied a basal feed mixture was used, which consisted of the following:

	Parts by weight
Ground yellow corn	500
Wheat bran	245
Rolled oats	150
Alfalfa-leaf meal	55
Total	950

The basal diet, which contained no protein supplement, varied somewhat from year to year. Its composition is given in table 1.

The minor variations in the composition of the basal diet broadened the scope of the investigation in that the tests included slightly different diets of the type used by investigators and in commercial poultry establishments. During the course of a particular year, however, the basic conditions of the experiment and of environmental factors were uniform for all pens involved in the comparative studies. Thus all results within a year are closely comparable, whereas those among the different years are only approximately comparable.

TABLE 1.—*Variations from year to year in composition of the basal diet which contained no protein supplement*

Constituent	Parts by weight		
	First year	Second year	Third year
Basal feed mixture	95.0	93.6	89.9
Ground limestone	2.5	5.4	4.0
Steamed bonemeal	1.5	.0	5.1
Anhydrous sodium sulphate	.5	.5	.5
Common salt	.5	.5	.5
Total	100.0	100.0	100.0

THE PROTEIN SUPPLEMENTS

Each of the other diets contained 20 parts, per 100 parts, of one of the packing-house byproducts used or of the meat-fish-milk protein supplement. The quantities of basal feed mixture, ground limestone, and steamed bonemeal were so adjusted that the diets contained the desired percentage of calcium and phosphorus.

In order to insure an adequate supply of vitamins A and D, 2 parts by weight of cod-liver oil were added to each 100 parts of the diets just before they were fed.

The protein supplements used in compounding the individual diets are described below. In 1932 and 1933 the Institute of American Meat Packers prepared a number of special products for use in this study. These are indicated by the letters "I. A. M. P." placed immediately after the name of the supplement.

Meat-fish-milk.—This supplement, which is not a packing-house byproduct but which was used in these experiments as a positive control, consisted of 40 percent of desiccated meat meal (described below), 35 percent of whitefish meal, and 25 percent of dried buttermilk. The whitefish meal was a commercial product prepared from the offal of the North Atlantic fishing industry by the vacuum-drying process. The dried buttermilk was the usual commercial product commonly used in feeding poultry.

Desiccated meat meal.—This is a special meat meal regularly prepared for the Bureau of Animal Industry, and is not available commercially. The material used in its preparation is the flesh of condemned carcasses of various kinds. It is processed in much the same manner as ordinary meat meals. It differs from the latter, however, in that it contains little bone, and its protein content is usually between 75 and 80 percent and its ash content usually less than 7.5 percent.

Ground, dried, lean meat (I. A. M. P.).—Only lean meat was used in making this supplement. It was a high-grade, ground, dried-meat product and was suitable for human consumption.

All-beef scrap no. 1 (I. A. M. P.).—This was a product such as is made for commercial purposes; it contained, however, no added high-protein materials such as blood meal and stick. The materials used in its preparation were: Carcasses, livers, spleens (known commercially as melts or milts), skulls (heads from which muscle tissue, skin, tongues, and brains were removed), beef rennets, tripe trimmings, hashed pecks (a packing-house term for finely chopped manyplies, the tissues of the third stomach of the ruminant), and beef-cutting scrap. No definite formula was used.

Meat-and-bone meal (55 percent protein) (I. A. M. P.).—This product was prepared from the meat-and-bone meal described below, with the addition of enough blood meal (about 25 percent) to bring the protein content to approximately 55 percent. The resulting product contained about 20 percent of blood meal.

Meat-and-bone meal containing no blood meal, stick, or other material added for raising the protein content (I. A. M. P.).—Skulls, livers, spleens, beef rennets, tripe trimmings, and hashed pecks were used in preparing this product.

Blood meal and stick (I. A. M. P.).—This consisted of a mixture of blood meal and stick in the proportions in which they commonly have been used in the manufacture of meat-and-bone meals of a relatively high protein content.

All-beef scrap no. 2 (I. A. M. P.).—This product was prepared from the same materials that were used in preparing all-beef scrap no. 1 (I. A. M. P.). In all-beef scrap no. 2, the following definite formula was used:

	Percent
Carcasses	20
Livers	10
Spleens	10
Skulls	10
Beef rennets	15
Tripe trimmings	10
Hashed pecks	15
Beef-cutting scrap	10
Total	100

The materials were mixed and then cooked in a vacuum (−20 to −26 inches of mercury) for 4 hours at a maximum temperature of 178° F.

All-beef scrap no. 2A (I. A. M. P.).—This product was the same as all-beef scrap no. 2 except that the materials were cooked in a vacuum (−21 to −25 inches of mercury) for 6 hours at a maximum temperature of 178° F.

All-beef scrap no. 2B (I. A. M. P.).—This product was the same as all-beef scrap no. 2 (I. A. M. P.) except that the materials were cooked in a vacuum (−20 to −24 inches of mercury) for 16 hours at a maximum temperature of 178° F.

All-beef scrap no. 2C (I. A. M. P.).—This product also was the same as all-beef scrap no. 2 (I. A. M. P.) except that the materials were cooked at atmospheric pressure for 8 hours at a maximum temperature of 200° F.

Mixture of 80 percent of all-beef scrap no. 2 and 20 percent of steam-dried blood meal.

Mixture of 80 percent of all-beef scrap no. 2 and 20 percent of spray-dried blood meal.

Mixture of 80 percent of all-beef scrap no. 2, 10 percent of steam-dried blood meal, and 10 percent of liquid stick.

Mixture of 80 percent of all-beef scrap no. 2, 10 percent of spray-dried blood meal, and 10 percent of liquid stick.

A commercial meat-and-bone meal.—This product was purchased on the open market.

Liquid stick (I. A. M. P.).—Liquid stick is the concentrated liquor from the steam rendering of fatty animal tissue. It consists largely of water-soluble compounds, including many of the products resulting from the hydrolysis of protein. This byproduct is not used as such by the poultryman; nevertheless, it was studied because it is an ingredient of some of the meat byproducts that he uses.

The Institute of American Meat Packers supplied the two kinds of blood meal used.

METHOD OF DETERMINING THE VITAMIN G CONTENT OF THE PROTEIN SUPPLEMENTS USED IN 1933–34

The materials supplied by the Institute of American Meat Packers for use in the 1933–34 experiment included two types of blood meal—steam-dried and spray-dried—and four lots of all-beef scrap which had been manufactured under different, but carefully controlled, conditions. Accordingly, these materials were assayed for vitamin G in order to ascertain whether or not the method of drying, in the case of the blood meals, or the temperature and length of time used in processing, in the case of the all-beef scraps, had affected the vitamin G content.

The assays were made by means of the rat-growth method. The procedure followed was essentially the same as that used in earlier work reported by Ellis, Miller, Titus, and Byerly.[4] The basal diet fed to the rats consisted of 20 percent of alcohol-extracted casein, 65 percent of partially dextrinized cornstarch, 4 percent of mineral mixture, 1 percent of agar-agar, and 10 percent of lard. Vitamins A and D were supplied by cod-liver oil, which was fed separately. A Lloyd's reagent adsorbate prepared from an aqueous extract of rice polish was added as a source of vitamin B. This adsorbate was comparable in vitamin B potency to the international vitamin B standard. A control group of rats was fed the basal diet, and other groups were fed the same diet and were given each day weighed quantities of the packing-house byproducts. The gains in weight of the rats receiving the packing-house byproducts were corrected for the change in weight of the rats in the control group and were then used for computing the vitamin G content of the materials being assayed.

[4] ELLIS, N. R., MILLER, D., TITUS, H. W., and BYERLY, T. C. EFFECT OF DIET ON EGG COMPOSITION. III. THE RELATION OF DIET TO THE VITAMIN B AND VITAMIN G CONTENT OF EGGS, TOGETHER WITH OBSERVATIONS ON THE VITAMIN A CONTENT. Jour. Nutrition 6:243–262, illus. 1933.

EXPERIMENTAL RESULTS

The results of the vitamin G assays made on the packing-house byproducts used during the fifth year of the investigation are given in table 2. The lack of any significant difference in vitamin G content among the four lots of all-beef scrap indicates that the temperature used and the length of time the lots were cooked had no marked effect on the vitamin G content of this product. Furthermore, according to the data presented in this table, the liquid stick used was a fairly good source of vitamin G inasmuch as it contained approximately as much as the different lots of all-beef scrap. These data also indicate that the method of drying the blood meal had no marked effect on the vitamin G content of this product.

TABLE 2.—*Vitamin G content of packing-house byproducts used as protein supplements in 1933–34*

Product	Rats	Level fed daily	Average gain in weight during—		Vitamin G per gram (approximate)
			8 weeks	1 week	
	Number	*Grams*	*Grams*	*Grams*	*Units*
All-beef scrap no. 2 (cooked 4 hours at maximum temperature of 178° F.)	7	0.5	38.3±2.1	4.8	3
All-beef scrap no. 2A (cooked 6 hours at maximum temperature of 178° F.)	7	.5	40.1±1.4	5.0	3
All-beef scrap no. 2B (cooked 16 hours at maximum temperature of 178° F.)	7	.5	46.4±2.7	5.8	3
All-beef scrap no. 2C (cooked 8 hours at maximum temperature of 200° F.)	7	.5	41.9±0.9	5.2	3
Liquid stick	7	.5	37.9±3.5	4.7	3
Steam-dried blood meal	7	.5	18.7±1.8	2.3	1.5
Spray-dried blood meal	7	.5	16.9±2.5	2.1	1.5

The pertinent data obtained in the chicken-feeding experiments are summarized in table 3.

EGG PRODUCTION AND FEED CONSUMPTION

As a measure of egg production, it is customary to use the figure obtained by multiplying the total number of eggs produced by a bird, or a flock, by 100 and dividing the result by the corresponding number of bird days. This figure is referred to as the percentage of egg production. Thus, if each bird in a flock lays one egg per day during a given period, the egg production for that period is 100 percent. When mortality in the flock is low, this measure may be fairly reliable, but when mortality is heavy, it cannot be depended on.

A more dependable measure of egg production is to adjust the average number of eggs laid for the number of bird days by the method of covariance analysis and then multiply the adjusted average by 100 and divide the result by the average number of bird days. When this procedure is followed, it is possible to obtain also a good estimate of the standard error of the percentage of egg production and thus have a statistical measure of its reliability.

Because of the extensive use of the first method among investigators and to provide comparisons, the percentage of egg production of the birds on the different diets was calculated by both methods, and the results are given in table 3. The values obtained by the two methods agree rather well except in the case of the diets fed during 1932–33, when the mortality was heavy.

TABLE 3.—*Data on protein supplements, fed to Rhode Island Red pullets, in relation to production and hatchability of eggs, live weight, and feed consumption*

Protein supplement	Protein content of—		Pullets at beginning of experiment	Total bird days	Total bird days as percentage of possible number	Live weight of pullets			Feed consumed—		Egg production		Average egg weight	Second-week embryonic mortality	Hatchability of fertile eggs
	Protein supplement	Diet				Average initial	Average maximum	Average final	Per bird (336 days)	Per egg	100× total eggs÷total bird days	Adjusted by covariance analysis			
	Percent	*Percent*	*Number*	*Number*	*Percent*	*Grams*	*Grams*	*Grams*	*Kilograms*	*Kilograms*	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
FIRST YEAR (1929–30)															
None		11.6	40	13,177	98.0	1,722	2,693	2,121	40	0.38	31.2	31.7±2.1	55.6	5.7	70.6±3.3
Meat+fish+milk	61.5	20.7	40	13,186	98.1	1,728	2,804	2,475	43	.30	42.7	43.0±2.1	58.2	5.8	72.7±1.9
Desiccated meat meal	72.4	23.8	40	12,891	95.9	1,770	2,757	2,466	44	.31	41.5	42.1±2.1	57.8	5.	70.1±2.7
SECOND YEAR (1930–31)															
None		11.6	25	7,832	93.2	2,034	2,350	1,978	36	0.34	31.5	31.4±1.9	53.4	18.1	52.2±6.2
Meat+fish+milk	59.3	20.7	15	4,947	98.2	1,644	2,432	2,272	42	.25	53.2	52.8±2.4	56.9	1.5	73.8±3.6
THIRD YEAR (1931–32)															
None		11.7	25	5,568	65.0	1,670	2,330	2,016	38	0.33	26.3	27.4±4.4	54.5	8.5	63.9±8.2
Meat+fish+milk	59.6	21.3	25	7,191	85.6	1,660	2,399	2,209	36	.29	35.0	34.2±4.4	55.8	2.1	68.1±3.3
FOURTH YEAR (1932–33)															
None		11.8	25	5,703	67.9	1,940	2,288	2,019	35	0.36	19.9	19.8±3.5	53.0	6.0	76.5±8.7
Meat+fish+milk	58.8	20.9	25	5,828	69.4	1,917	2,378	2,194	35	.34	24.6	23.1±3.5	56.6	1.4	78.9±10.2
Ground, dried, lean meat	61.3	21.5	50	12,028	71.6	1,915	2,346	2,075	38	.31	32.6	29.2±2.5	55.3	5.7	65.7±3.6
All-beef scrap (no. 1)	59.0	21.4	50	9,851	58.6	1,934	2,389	1,994	38	.32	28.8	25.2±2.5	54.5	1.1	80.6±1.0
Meat-and-bone meal (55 percent protein)	55.2	20.4	50	9,464	56.3	1,920	2,349	2,047	36	.31	27.0	23.8±2.5	55.8	2.5	76.5±5.8
Meat-and-bone meal containing no added blood meal or stick	48.3	19.5	50	9,676	57.6	1,932	2,354	2,081	37	.31	28.1	24.8±2.5	53.6	4.0	70.6±2.5
Blood meal and stick	80.6	24.4	50	10,813	64.4	1,936	2,263	2,118	36	.30	30.2	26.7±2.5	53.7	18.9	34.2±4.6

FIFTH YEAR (1933-34)

All-beef scrap no. 2; cooked 4 hours at maximum temperature of 178° F.	56.2	20.0	38	11,122	87.1	2,085	2,546	2,100	36	0.33	29.2	29.7±2.5	55.5	1.9	76.5±2.8
All-beef scrap no. 2A; cooked 6 hours at maximum temperature of 178° F.	57.3	20.3	38	10,900	85.4	2,031	2,407	1,898	33	.34	25.6	26.7±2.5	53.8	1.9	81.8±2.7
All-beef scrap no. 2B; cooked 16 hours at maximum temperature of 178° F.	56.4	19.7	38	11,193	87.7	2,070	2,483	2,052	35	.35	26.6	27.4±2.5	56.4	1.9	73.5±2.7
All-beef scrap no. 2C; cooked 8 hours at maximum temperature of 200° F.	61.3	21.2	38	10,187	79.8	2,091	2,503	2,111	36	.34	25.8	27.2±2.5	55.1	1.9	76.7±5.6
All-beef scrap no. 2+steam-dried blood meal	61.0	20.7	38	10,643	83.6	1,995	2,421	2,085	35	.33	28.0	28.8±2.5	54.9	.9	70.0±3.2
All-beef scrap no. 2+spray-dried blood meal	61.7	20.1	38	10,228	80.1	2,029	2,455	2,015	36	.36	23.4	25.2±2.5	56.7	1.8	70.9±4.3
All-beef scrap no. 2+steam-dried blood meal+liquid stick	58.6	20.6	16	4,896	91.1	2,033	2,513	1,965	35	.31	31.3	31.5±3.9	56.0	.9	67.6±5.4
All-beef scrap no. 2+spray-dried blood meal+liquid stick	58.9	20.8	16	4,333	80.6	2,005	2,529	2,087	32	.30	26.9	28.3±3.9	57.2	1.2	72.5±6.4
A commercial meat-and-bone meal	49.1	19.7	22	6,502	88.0	2,148	2,442	1,904	36	.36	26.9	27.6±3.3	55.9	3.2	70.9±8.5
Liquid stick	56.2	20.4	22	6,610	89.4	2,042	2,452	2,125	38	.38	26.7	27.4±3.3	52.7	6.4	53.8±6.8

The data in table 3 show that in all cases the average egg production and egg weight per bird for the diets containing the meat-fish-milk supplement were greater than for the diets containing no supplement. Also, in the 1932–33 experiment, when it was possible to make direct comparisons between the diet containing the meat-fish-milk supplement and the diets containing the packing-house byproducts, the latter diets produced more eggs per bird than did the former diet. In average egg weight per bird, on the other hand, during the same year the meat-fish-milk supplement gave somewhat better results than did the packing-house products. However, the difference was not significant in either case, nor did the results show any significant differences among the diets containing the various packing-house byproducts. Furthermore, in 1933–34 the test with the all-beef scrap showed that the period and temperature of cooking and the addition of blood meal and liquid stick had no consistent effect on number or weight of eggs.

The smallest eggs per bird were produced on the diet containing the liquid-stick supplement, and comparatively small eggs were produced by birds receiving blood meal and stick and by those receiving meat-and-bone meal containing no added blood meal or stick. However, the eggs produced on most of the supplements tested were of satisfactory commercial weight. The majority weighed 55 g or more, or approximately $1^{11}/_{12}$ ounces.

Table 3 shows that the birds receiving the meat-fish-milk supplement consumed less feed per egg than the birds receiving no protein supplement. However, in 1932–33, when most of the comparisons between the diet containing meat-fish-milk and those containing the packing-house supplements could be made, the birds on the former diet consumed somewhat more feed per egg than those on the latter diets. In the tests with all-beef scrap during the fifth year, there appeared to be no consistent differences in feed consumption as the result of different periods and temperatures of cooking and the addition of blood meal and liquid stick. The pullets receiving the liquid stick as the protein supplement in the diet required the largest quantity of feed per egg produced.

HATCHABILITY

The methods developed by Hendricks [5] for computing the percentage of fertile eggs that hatch and the standard error of this percentage were used in obtaining the values given in the last column of table 3. The data show that the hatchability of eggs produced on the diets containing the meat-fish-milk supplement was greater than on the diets containing no supplement, but the difference was statistically significant in only one case. In general, however, the data indicate that the former diets are definitely superior to the latter for the production of hatchable eggs. Also, in the cases in which direct comparisons were possible, the diets containing the meat-fish-milk supplement were superior in hatchability to those containing the packing-house byproducts except for the diet containing all-beef scrap no. 1.

When compared among themselves, the diets containing the packing-house byproducts showed wide differences in their effect on

[5] HENDRICKS, W. A. THE STATISTICAL TREATMENT OF HATCHABILITY DATA. Poultry Sci. 14: 365–372 1935.

hatchability. In the experiment conducted in 1932–33, the use of ground, dried, lean meat as a protein supplement led to the production of a few more eggs than did the use of the all-beef scrap, but the hatchability on the diet containing the former was significantly less than that on the diet containing the latter. The use of blood meal and stick also led to the production of a few more eggs than did the use of most of the other supplements, but the hatchability was less than half as good as when the other supplements were used.

In 1933–34, the use of the all-beef scrap no. 2A, which was cooked for 6 hours, resulted in a significantly higher hatchability of the fertile eggs set than did the all-beef scrap no. 2B, which was cooked for 16 hours. In the case of all-beef scrap nos. 2 and 2C, which were cooked for 4 and 8 hours, respectively, the differences in hatchability were not statistically significant. When liquid stick was used as the protein supplement, the hatchability of the eggs was distinctly decreased. Additions of blood meal and stick to the all-beef scrap seemed to have a slightly deleterious effect on hatchability, but the results were not uniformly consistent.

SECOND-WEEK EMBRYONIC MORTALITY

The data on second-week embryonic mortality show some noticeably wide variations. In the case of the diets containing the meat-fish-milk supplements, this mortality was comparatively small, being less than 2.5 percent during 3 of the 4 years in which this supplement was used. On the other hand, in no instance was the second-week embryonic mortality less than 5.7 percent in the case of the unsupplemented diets, and in one instance it was 18.1 percent.

For those diets which contained the packing-house byproducts, the range in second-week embryonic mortality was rather wide, apparently because of the diverse nature of the byproducts. The mixture of blood meal and stick, which was fed in 1932–33, resulted in the greatest second-week embryonic mortality of any of the byproducts used. Liquid stick and the meat-and-bone meal purchased on the open market in 1933–34 apparently caused an increase in second-week mortality, as compared with the other products used the same year. Another product which apparently caused an increase in the number of embryo deaths during the second week of incubation was the ground, dried, lean meat used in 1932–33. All lots of all-beef scrap, regardless of the length of time they were cooked or the temperature used, were associated with low mortality during the second week of embryonic life.

In several instances the low hatchabilities were attributable in part to other causes than a high second-week embryonic mortality. This is well illustrated in figure 1, in which the daily mortality, expressed as percentage of fertile eggs set, is plotted against the length of time the eggs had been incubated. The distribution of embryonic mortality, when diets containing all-beef scrap were fed, was approximately normal, that is, it was very similar to that observed when the birds are well fed and are given access to range. In the case of the diet containing blood meal and stick and the one containing liquid stick, the mortality was excessive throughout, and there was not only an increased second-week mortality but an increased first- and third-week mortality as well. The increase in the number of deaths that

occurred during the third week of incubation in the eggs produced with liquid stick as a protein supplement was very pronounced.

LIVE WEIGHTS AND HEALTH OF PULLETS

It is apparent from the data in table 3 that a protein supplement in the diet had some influence on live weight. In all cases, the average maximum live weight attained on the diets containing the meat-fish-milk protein supplement was greater than that attained on the diets containing no protein supplement. Apparently, the packing-

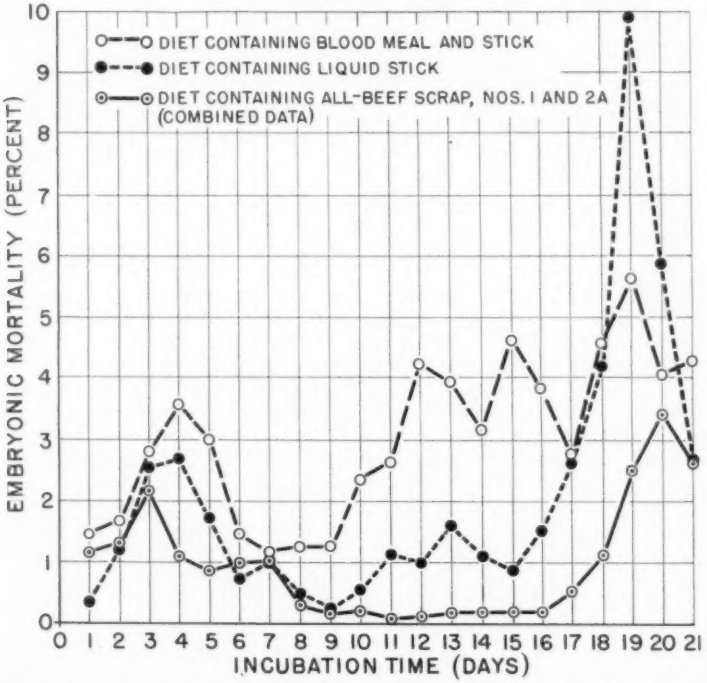
DIET CONTAINING BLOOD MEAL AND STICK
DIET CONTAINING LIQUID STICK
DIET CONTAINING ALL-BEEF SCRAP, NOS. 1 AND 2A (COMBINED DATA)
EMBRYONIC MORTALITY (PERCENT)
INCUBATION TIME (DAYS)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
0 1 2 3 4 5 6 7 8 9 10

FIGURE 1.—Comparison of mortality of embryos in eggs produced on diets containing blood meal and stick, liquid stick, and all-beef scrap.

house byproducts permitted the birds to reach about the same average maximum live weight as the meat-fish-milk supplement. Although blood meal and stick gave a somewhat lower average maximum live weight than any other diet fed in the fourth year of the experiment, the average final live weight of the birds receiving this supplement was comparatively high.

The average live weight of a flock of birds toward the end of their first laying year is a fair measure of the ability of the diet to maintain live weight in spite of the demands for egg production. In all cases, within the same year, the diets containing the meat-fish-milk supplement were somewhat better than those containing no supplement as well as those containing the packing-house byproducts,

although in 1932–33 the differences were not marked. The results showed no marked differences among the various packing-house byproducts in either average maximum or final live weights. During the last year the final weights in several instances were slightly lower than the initial weights, but both maximum and final live weights attained on all the packing-house byproducts compared favorably with previous results obtained with the positive control, allowance being made for relative initial weights of the pullets.

During the first year, 1929–30, the mortality on all three diets was appreciably lower than is ordinarily observed during the first 336 days after pullets are placed in laying quarters. In 1930–31, however, the disease known as lymphomatosis was first observed among the flocks of chickens on the experiment farm, and in some of the experimental pens there was a marked increase in mortality. During the next 2 years sickness and mortality rapidly increased. In 1933–34 the disease showed a definite tendency to decrease, but it was still somewhat greater than is ordinarily observed in laying flocks. Affected birds were promptly removed from all experimental pens, and the methods of calculation eliminated any appreciable effects of the disease factor on the significant results of the study.

DISCUSSION

One of the most striking results of these experiments was the observation that although several diets supported relatively good egg production, many of the eggs failed to hatch. This appeared to be especially true in the case of diets which contained (1) ground, dried, lean meat, (2) blood meal and stick, and (3) liquid stick. On the other hand, the experiments clearly showed the possibility of preparing byproducts which not only will support good egg production but which will lead also to the production of eggs that hatch well.

In searching for the reason for this difference among packing-house byproducts, the writers found that the nature of the raw materials used in their preparation was a very important factor. But apparent quality from a human-food standpoint did not necessarily mean a satisfactory supplement for the diet of pullets in a breeding flock. For example, the ground, dried, lean meat was a product of excellent quality and considerable care had been taken in its manufacture, yet, so far as hatchability was concerned, it was decidedly inferior to all-beef scrap no. 1, which was made from materials largely unsuitable for human consumption. The liquid stick and the mixture of blood meal and stick were products of a very different kind from those just mentioned, in that stick contains a large percentage of water-soluble compounds, many of which result from the hydrolysis of protein, and no special care is used in its preparation. These two supplements caused an increased second-week embryonic mortality and definitely decreased hatchability.

In the last experiment of the series, the addition of blood meal, or of mixtures of equal parts of blood meal and liquid stick, to the all-beef scrap had no consistent effect on egg production or hatchability, in spite of the fact that both the liquid stick and a mixture of blood meal and stick, when used as the sole protein supplement, decreased the hatchability quite markedly.

So far as the effect of temperature and time of processing were concerned, the few data obtained indicate that cooking temperatures

not greater than 200° F., maintained for not more than 8 hours, were without material effect on egg production or hatchability. There is some indication that prolonged cooking for 16 hours or more, even at a temperature of 178°, may have an unfavorable effect on hatchability.

According to Ellis, Miller, Titus, and Byerly [6] and unpublished data obtained by them, the basal diets used in this study were moderately rich in vitamins B and G. Hence, there is little likelihood that vitamin G was a limiting factor in any of the diets studied. Furthermore, according to the data presented in table 2, the liquid stick used in 1933–34 was a fairly good source of vitamin G inasmuch as it contained practically as much as the different lots of all-beef scrap used in that experiment. Also, these data indicate that the two kinds of blood meal contained about one-half as much vitamin G as the sample of stick and the different lots of all-beef scrap. Therefore, it is evident that the increase in second-week embryonic mortality observed in feeding the diets containing liquid stick and a mixture of blood meal and stick was not due to a deficiency of vitamin G.

Nestler, Byerly, Ellis, and Titus [7] have presented evidence that the basal feed mixture used in these experiments lacks some factor necessary for good hatchability and that this factor is relatively abundant in dried liver and green grass and present to some extent in dried buttermilk. They concluded that dried whey, which is a good source of vitamin G, is not a good source of this factor. Apparently, this factor was well supplied by the several lots of all-beef scrap which were used and which contained some liver.

The value of liver in nutrition has become more and more appreciated during the last decade. This has created a demand for it that has lead to an increase in its cost, with the result that large quantities which formerly were used in making meat scrap and similar products are now sold as dried liver. It would be a good practice for manufacturers to state the liver content of such of their products as do contain liver.

Barnum [8] concluded that vitamin E may be a limiting factor affecting the hatchability of eggs produced on certain diets and that one manifestation of vitamin-E deficiency in chickens is an increased first-week embryonic mortality. In the present experiments, when liquid stick and a mixture of blood meal and stick were used as the protein supplements, there was a definite increase in first-week embryonic mortality (fig. 1). This suggests that the stick and mixture of blood meal and stick may have caused the destruction of some of the vitamin E originally present in the basal feed mixture.

The high third-week embryonic mortality observed in the case of the diets containing stick and a mixture of blood meal and stick was not observed in the case of any of the other diets. The reason for this rather pronounced effect is not known.

SUMMARY AND CONCLUSIONS

A study was made of the effects of packing-house byproducts in the diet of Single-Comb Rhode Island Red pullets on the production and hatchability of eggs. Data were likewise obtained on the effect

[6] ELLIS, N. R., MILLER, D., TITUS, H. W., and BYERLY, T. C. See footnote 4.

[7] NESTLER, R. B., BYERLY, T. C., ELLIS, N. R., and TITUS, H. W. A NEW FACTOR, NOT VITAMIN G, NECESSARY FOR HATCHABILITY. Poultry Sci. 15: 67–70. 1936.

[8] BARNUM, G. L. THE VITAMIN E CONTENT OF EGGS AS RELATED TO THE DIET OF THE HEN AND TO HATCHABILITY. Jour. Nutrition 9: 621–635. 1935.

of such products on live weight, feed consumption, egg weight, and embryonic mortality.

The packing-house byproducts used in the tests included various kinds of meat meal, meat-and-bone meal, beef scrap, blood meal, and stick. These were tested as individual products and several were used in various combinations. The experiment likewise involved comparisons of the effects of different methods of cooking all-beef scrap.

In general, the egg production of birds on diets containing packing-house byproducts was materially higher than that of birds on the basal diet containing no supplement. Egg production resulting from the feeding of packing-house byproducts was somewhat higher than on diets containing a meat-fish-milk supplement known to be efficient in this respect.

In general, the packing-house byproducts, besides supporting reasonably good egg production, resulted in satisfactory hatchability, ranging in most cases between 70 and 80 percent. Decreased hatchability, when encountered, was rather closely associated with an increased second-week embryonic mortality. In the case of the diets containing a specially prepared all-beef scrap, this second-week embryonic mortality was almost negligible.

Liquid stick and a mixture of blood meal and stick led to an increase of embryonic mortality throughout the incubation period and the increase was especially noticeable during the third week.

In their effect on live weight, the packing-house byproducts were about equal. All the byproducts permitted the birds to reach approximately the same average maximum live weight as the meat-fish-milk supplement and the weights of the birds were reasonably well maintained to the end of the experiment.

In their effect on feed consumption per egg produced, the packing-house byproducts were slightly more efficient than the meat-fish-milk supplement. In effect on egg weight, this supplement produced slightly heavier eggs than the packing-house byproducts tested. Most of the packing-house byproducts enabled the birds to produce eggs of satisfactory weight and size. The birds receiving liquid stick produced the smallest eggs. Those receiving a mixture of blood meal and stick and those receiving meat-and-bone meal containing no added blood meal or stick produced relatively small eggs.

The studies indicate that the materials used in making good meat scrap and similar products are relatively more important than the temperature and the time of processing so long as the temperature does not exceed 200° F. or the time of processing 8 hours. Higher temperatures were not studied.

The experiments indicate the commercial possibility of producing excellent packing-house byproducts from the point of view of good hatchability as well as good production. A formula which was found to produce generally satisfactory results consisted of: Carcasses 20 percent, livers 10, spleen 10, skulls 10, beef rennets 15, tripe trimmings 10, hashed pecks 15, and beef-cutting scrap 10.

In general, the experiments confirm previous evidence regarding the value of protein supplements of animal origin, and direct attention to opportunities for improving such supplements by the development and use of suitable formulas and controlled methods of manufacture.

A SEEDLING WILT OF BLACK LOCUST CAUSED BY PHYTOPHTHORA PARASITICA[1]

By EDMUND B. LAMBERT, *associate pathologist, Division of Mycology and Disease Survey*, and BOWEN S. CRANDALL, *assistant scientific aide, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

Large increases in the number of black locust (*Robinia pseudoacacia* L.) seedlings grown in recent years have brought into prominence diseases of this species hitherto overlooked or considered of minor importance. Such a disease developed in 1933 in a Virginia nursery. Many beds were a total loss although resown several times. Toward the end of the epidemic, field observations by Carl Hartley indicated that the disease was a top wilt of plants whose roots still appeared sound. In view of these observations the studies reported in this paper were begun in the fall of 1933.

SYMPTOMS

Opportunities to observe the symptoms of the disease under various conditions have been afforded by epidemics in the field in 1934 and 1935 and by numerous cases resulting from artificial inoculations in the greenhouse.

The severest damage and the most characteristic symptoms of the disease develop in seedlings 1 to 3 weeks old. In seedlings of this age all of the plants may wilt in patches several feet in diameter or even throughout entire beds. Usually the first visible sign of the disease is a slight drooping or curling of the cotyledons (fig. 1, *B*). It is followed, usually in less than a day, by sudden wilting, collapse, and shriveling of the entire upper portion of the seedling (fig. 1, *C* and *D*). Beds that appear to be in fine condition in the evening may be found with large patches of wilted seedlings when examined the following morning.

A day after the wilt develops to the stage indicated in figure 1, *D*, the seedlings have a somewhat conical appearance due to the withered cotyledons and first foliar leaves clinging to the erect stem. At this stage the base of the stem is still turgid. Typically, there is no evidence of a lesion on the stem at the soil surface or of fungus invasion of the roots. The entire seedling withers within a few hours. If beds are not observed regularly, the extent of the loss is not realized, since young wilted seedlings disintegrate rapidly, often almost entirely disappearing in 4 to 5 days.

A preemergence rotting of seedlings also seemed to be a part of the disease complex both in the greenhouse and in the field; but this is not so easy to diagnose nor so apparent in affected beds as the top wilt.

[1] Received for publication Mar. 31, 1936; issued October, 1936.

[2] The writers wish to express their appreciation to Carl Hartley, principal pathologist, Division of Forest Pathology, for placing at their disposal the results of his observations during the summer of 1933, and for counsel during the course of the investigation.

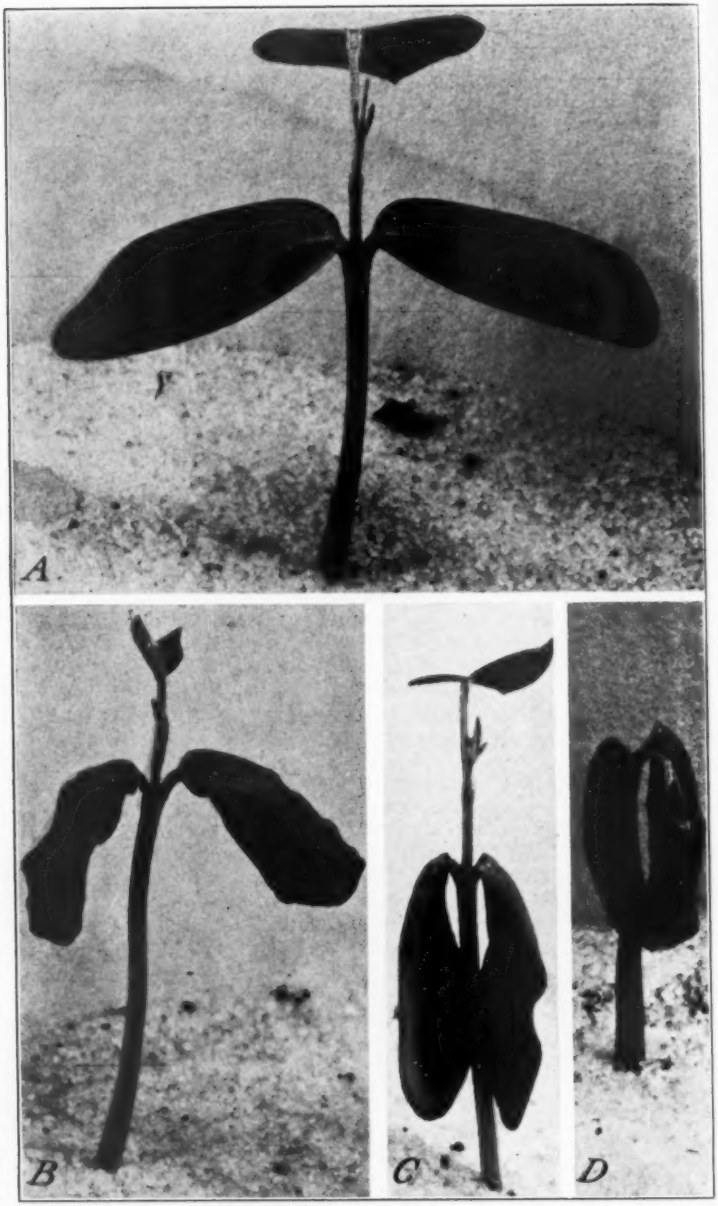

FIGURE 1.—Typical symptoms of wilt disease affecting seedlings 5 days old: *A*, Healthy seedling; *B*, first visible stage of wilt; *C*, cotyledons collapsed; *D*, cotyledons and first foliar leaves wilted but lower stem and roots not yet affected.

After the seedlings are 3 or 4 weeks old, succulent new shoots may wilt rather suddenly here and there in a manner quite similar to the wilting of young seedlings, but the disease is clearly not systemic and is rarely fatal. As the leaves grow older the diseased areas become more localized. Necrotic areas from 5 to 10 mm across may develop on leaves that are otherwise healthy and continue to function. Occasionally leaves will suddenly shrivel as a result of girdling of the petioles. Darkened and sharply defined lesions develop on older stems. These lesions are found at any point on the stem or petiole and frequently remain small and sharply limited without further spread.

Field observations have shown that outbreaks of the disease usually follow a period of cloudy or rainy weather. Typical symptoms usually develop 2 to 4 days after an overnight rain. These observations have been corroborated by studies on the life cycle of the *Phytophthora* causing the disease. During extended periods of cool wet weather, young seedlings may be extensively invaded by fungus hyphae. In such cases, the first symptoms are manifested as soon as the sun begins to dry off the beds, when thousands of seedlings may wither within a few hours.

When diagnosing the disease in beds of young seedlings, care should be taken not to confuse it with the root rot type of damping-off, or with the lesions at the level of the soil surface due to *Rhizoctonia*, *Pythium*, or possibly *Fusarium*.[3] *Phytophthora* rarely attacks the seedling roots or causes lesions at the soil level, although it is sometimes found mixed with *Rhizoctonia* in such lesions. Drought, heat injury, and the insect injury noted by Snyder and Lamb (*5*)[4] may also be sources of confusion. The surest diagnostic signs of the disease are the top wilt of young seedlings together with an erect turgid stem base and uninjured root.

ISOLATION AND INOCULATION EXPERIMENTS

Attempts to isolate a pathogen were begun in the greenhouse at Washington, D. C., in the fall of 1933. Soil was shipped in from the diseased beds of the Virginia nursery and sown with black locust seed. Pots containing young seedlings growing on this soil were incubated a few days in a moist chamber. Irregular stands were obtained owing to several causes which at first were rather confusing. However, there were a few wilted seedlings with drooping cotyledons characteristic of the disease as it appeared in the field. A *Phytophthora* was consistently isolated in practically pure culture from the upper parts of these seedlings. As in the field, this wilt affected the cotyledons and juvenile leaves first, then progressed downward into the petiole and upper part of the stem.

Preliminary experiments were made on potted seedlings in order to test the effect of adding the *Phytophthora* culture to uninfected greenhouse soil. It was apparent from these experiments that the *Phytophthora* when growing in the soil could cause a top wilt and, under favorable conditions, a preemergence damping-off.

The presumption was that the top wilt was caused by swarm spores distributed during the watering of the pots. To test this, an

[3] *Rhizoctonia* was repeatedly isolated from lesions at the soil surface both in the greenhouse and in the field. *Pythium myriotylum* Dresch. (identified by Charles Dreschler) was isolated from diseased seedlings in a North Carolina nursery. *Fusarium* was isolated from the roots of diseased seedlings.

[4] Reference is made by number (italic) to Literature Cited, p. 476.

experiment was made in which 16 seedlings growing in 4 pots were atomized with a water suspension of active swarm spores and a like number were atomized with pure water. The seedlings, which were at the stage of development at which they have cotyledons, a single large juvenile leaf, and small compound leaves (fig. 1, *A*), were then incubated in a moist chamber for 24 hours. Three days later all 16 inoculated seedlings were drooping in a manner typical of the top wilt, and *Phytophthora* was easily isolated from them. The seedlings sprayed with water and incubated at the same time remained healthy.

The evidence obtained from these experiments has been substantiated by numerous subsequent inoculations in the greenhouse, and by isolations made from diseased seedlings in nursery plantings in the summers of 1934 and 1935.

DESCRIPTION OF THE FUNGUS

In the course of the greenhouse studies and subsequent field studies, more than 50 isolates of *Phytophthora* were cultured from black locust seedlings. Although differences in cultural characters were exhibited by different isolates, all of the strains apparently belong to one species. They produce an abundant white aerial mycelium at room temperature on such standard media as oatmeal agar, corn-meal agar, lima bean agar, potato-dextrose agar, steamed rice, and steamed oatmeal. In corn-meal agar the mycelium is nodose and sterile for the first few days, but after 1 or 2 weeks sporangia are produced in moderate numbers. Typical sporangia are prominently papillate and broadly ovate, varying in size around an approximate average of 38μ by 45μ; they are produced in abundance 1 or 2 days after a bit of mycelium, growing on lima bean agar or in locust tissue, is transferred to water. In water they are as a rule nearly spherical; the ratio of length to width is about 1.2 to 1.0. Zoospores are produced in abundance in water cultures subjected to an occasional change of water and kept reasonably free from bacterial contamination. They become active within the sporangia, and swim out at first in a clump and later singly without any evidence of a vesicle. Chlamydospores, usually brown and mostly intercalary, may be found in abundance submerged in cultures on corn-meal agar after 2 weeks' growth at room temperature. They are usually thicker walled than the sporangia and average about 30μ in diameter. No sexual spores were observed, although attempts were made to produce them by crossing all available strains on oatmeal agar and corn-meal agar. The isolates tested have a comparatively high maximum-growth temperature; they continue to grow slowly for at least 4 days at 35° C.

The foregoing attributes indicate that the isolates of *Phytophthora* under consideration are referable to the composite species *P. palmivora* suggested by Leonian (*4*) or to the more limited concept expressed by Tucker (*6*) for *P. parasitica* Dast.[5]

PATHOLOGICAL HISTOLOGY

In order to study the progress of the pathogen within the host, seedlings were grown under aseptic conditions in test tubes and in-

[5] A culture of one of these isolates was submitted to C. M. Tucker, of the Missouri College of Agriculture, for identification. In his opinion, as expressed in correspondence, it is typical of *P. parasitica*.

oculated with pure cultures of *Phytophthora*. Hyphae were readily distinguished in freehand sections which had been fixed with Carnoy's solution and stained with orange G. Forty-eight hours after inoculation, when petioles of young seedlings were sectioned, the hyphae could be seen advancing in the phloem parenchyma just beneath the epidermal layer and between the cambium cells. At first the mycelium is predominantly intercellular, penetrating the cells with haustoria only (fig. 2). After the hyphae have

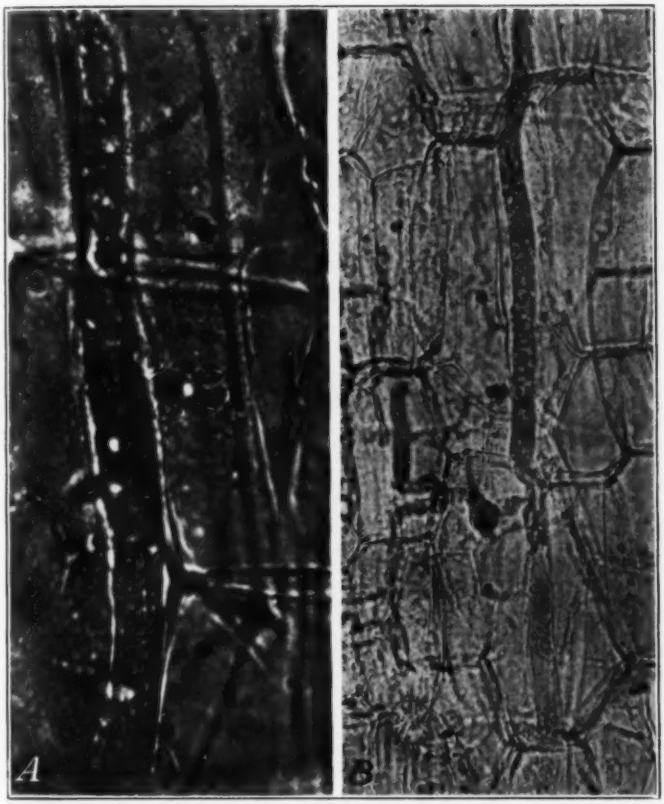

FIGURE 2.—Intercellular growth of hyphae of *Phytophthora parasitica* in phloem parenchyma of 2-week-old *Robinia* seedlings: *A*, Hypha alongside of a cell with a haustorium within the cell, × 1,450; *B*, hyphae following the intercellular spaces, × 350.

progressed a few millimeters, they begin to penetrate the cell walls and shortly thereafter become omnipresent regardless of cell type. Under these conditions, the tissue is soon softened and disorganized.

Experiments in which seedlings of different ages and different degrees of etiolation were sprayed with swarm spores indicate that the pathogen can cause extensive necrosis only in young, succulent host tissue. The sudden dying of numerous shoots frequently observed in the field in month-old plants is due to numerous simul-

taneous infections rather than to a systemic invasion of the plant. The collapse of large compound leaves is usually due to atrophy resulting from the girdling of the petiole by a comparatively small necrotic lesion, rather than to extensive invasion by the pathogen. When compound leaves wilt as a result of hyphal invasion the adja-

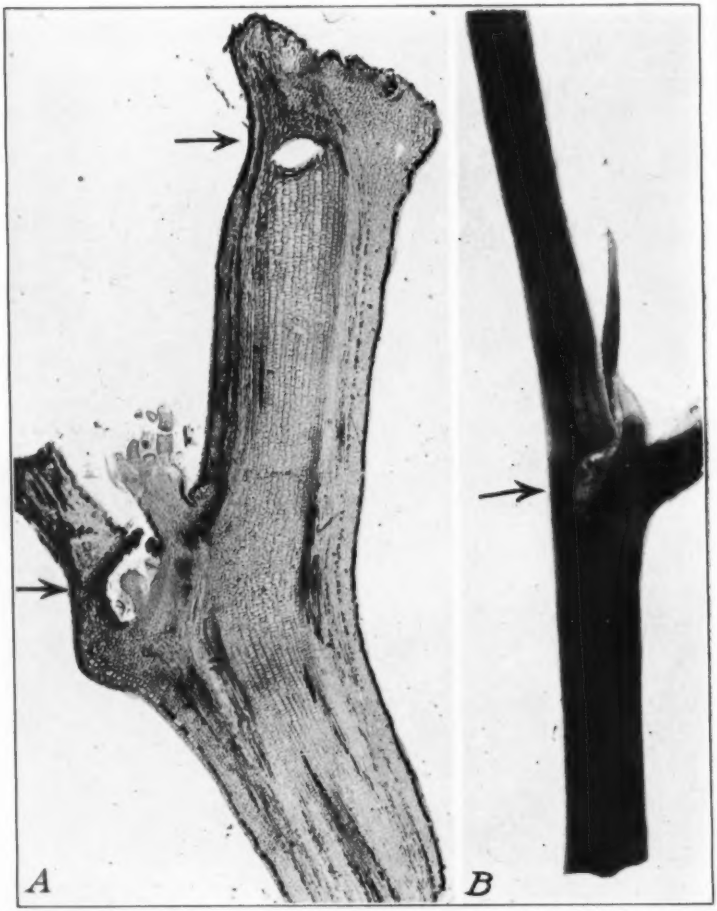

FIGURE 3.—Sections through point of attachment of compound leaf to stem of month-old *Robinia* seedling: *A*, Section stained with safranine and light green, showing result of hyphal invasion from terminal bud in tissue indicated by upper arrow, and the corked-off remains of an infected compound leaf at the point indicated by the lower arrow, × 19; *B*, cutaway view of the base of a normal compound leaf, showing the vascular tissue and cutinized layer tending to isolate the petiole from the bud, × 9.5.

cent buds remain healthy in a surprisingly large proportion of the cases. Examination of sections made through the point of attachment of the petioles of compound leaves suggests that this is due to the scarcity of parenchymatous connective tissue between the petioles and the adjacent buds (fig. 3).

EPIDEMIOLOGY AND GEOGRAPHIC DISTRIBUTION

Once the fungus is established in a nursery bed, it is evident that prolonged rains during seedling germination favor the spread of the disease. Sporangia and swarm spores are produced 5 or 6 hours after agar cultures are flooded with water. The swarm spores encyst in 5 to 20 minutes after emerging from the sporangia and germinate in 1 to 2 hours. Seedlings have been repeatedly infected with only 4 hours' incubation in a moist chamber after being sprayed with swarm spores. The whole process, therefore, of sporangia production, swarm-spore liberation, migration, encystment, germination, and penetration can easily take place during an overnight rain. Little is known of the resting phases of the life cycle. The fungus presumably lives through unfavorable conditions as chlamydospores, sporangia, dormant mycelium, or oospores in organic matter.

The known distribution of the disease is limited to four nurseries, one in Virginia, two in North Carolina, and one in Alabama. It seems probable, however, that it will soon be found in other places where large plantings of *Robinia* are placed in the natural geographic range of *Phytophthora parasitica*. Tucker (*6*) has assembled information pertinent to this question. He outlines the geographic distribution of this species as typically tropical or subtropical. He further points out that in the United States the species is most generally distributed in the Gulf and South Atlantic States, although it is also found in temperate regions with high humidity, particularly where summer temperatures are high.

Several published references to damping-off of *Robinia* seedlings have come to the attention of the writers. Of these, only the papers of Hartig (*2*), in Germany, and, according to Tucker (*7*), of Collinge (*1*), in England, ascribe the diseases under observation to *Phytophthora* sp. In both these cases, the *Phytophthora* was referred to the *P. cactorum* (L. and C.) Schroet. group rather than to the *P. palmivora-P. parasitica* group. Therefore, as far as the writers have been able to ascertain, there is no published account of a seedling disease of *Robinia* that can be identified with the disease described in this paper.

CONTROL MEASURES

The nature of the disease suggests the possibility of control by soil treatment or by a spray program.

Preliminary tests were made in the greenhouse to determine the effect on the prevalence of the disease of (1) several soil disinfectants, (2) a range of soil acidities, and (3) spraying with sulphur or copper sprays. Of the soil treatments tested, the acidification of the soil with aluminum sulphate appeared to be more promising than any of the disinfectants.

Spray tests were made by first spraying month-old seedlings with a fungicidal spray and later atomizing them at different intervals with active swarm spores of *Phytophthora parasitica*. In these tests protection was obtained with home-made bordeaux mixture (4–6–50), but not with Ansul sulphur spray. Good protection was maintained for at least 2 days after spraying with bordeaux, but infection was not prevented when swarm spores were applied 5 days after spraying, presumably because of the growth of new shoots from under the protective coatings.

In the summer of 1934, three separate series of experiments were made, each in triplicate, to compare the effects of different treatments, which were applied as (1) seed dusts, (2) soil treatments, and (3) sprays. The first series was made in a North Carolina nursery, and the second and third were made in the Virginia nursery where the disease was discovered. Little or no disease developed in the first series, and only a very irregular patchy epidemic developed in the second. In the third experiment, several buckets of soil from part of a bed badly diseased in the previous experiment were spread over the test beds before the treatments were applied. This, combined with suitable weather conditions, produced a uniform epidemic which killed nearly 100 percent of the seedlings in all of the untreated plots. The first two experiments made it possible to eliminate several obviously unsatisfactory treatments, but since these experiments were not very conclusive only the third is discussed in detail.

In the third series the seed treatments consisted of dusting the seed with zinc oxide, copper carbonate, and red copper oxide. The two soil treatments tested were as follows: Bordeaux dust was raked into the surface layer of soil at the rate of one-fourth ounce per square foot of bed space and aluminum sulphate was raked in dry at the rate of one-half ounce per square foot. At the rate applied, the aluminum sulphate increased the acidity of the soil on an average from pH 6.3 to pH 4.6.

The bordeaux spray was prepared by stirring 4 ounces of Orchard Brand mixture into 1 gallon of water. On plot 10 each application of the spray was made at the rate of three-fourths of a gallon to 100 square feet of bed space. Twice as much of the spray was applied each time to plot 12. Four applications of bordeaux spray were made; the first, 2 days after germination was noticed; the next, 2 days later; the next, 4 days later; and the last, 8 days later. The spray was applied at these intervals in order to keep the plants covered during the critical period. The first two applications, at 2-day intervals, kept the cotyledons covered; the application 4 days later covered the single juvenile leaf; and the application 8 days later covered the young compound leaves.

The seed used was furnished by the nursery. It appeared to be excellent seed, but scarification [6] was necessary to obtain good germination and a uniform time of seedling emergence. In field trials only 2 percent of the unscarified seed germinated, while 26.8 percent of the scarified seed germinated.

The plots were laid out in the order shown in table 1, except that all the plots designated by the letter "a" were grouped in one block, the "b" plots in another, and the "c" plots in another. Plots 10, 12, and 14 were not replicated. At the time the experiment was laid out there did not seem to be much hope of obtaining a uniform epidemic over all the plots, so the bordeaux spray trials (nos. 10 and 12), which seemed to be the most promising, and the copper carbonate seed-dusting treatment (no. 14) were crowded onto a section of one of the beds which had suffered 100-percent loss in the previous experiment. This space was too limited to permit replication.

[6] The seed was scarified by W. M. Hurst, of the Bureau of Agricultural Engineering, U. S. Department of Agriculture, in a barrel seed scarifier similar to that described by Hurst, Humphries, and McKee (*5*). The seed was scarified for 10 minutes with ¼- to ¾-inch gravel in the proportion of 5 pounds of gravel to 1 pound of seed.

The notes taken during the progress of the epidemic are summarized in table 1. The extensive wilting observed on September 11 occurred during a hot spell following a cool rainy period from September 7 to 10. (Almost identical conditions accompanied similar losses in the second experiment.) Three treatments were quite evidently effective in decreasing the percentage of wilted seedlings: Bordeaux dust in the soil, aluminum sulphate in the soil, and bordeaux spray. Both of the soil treatments were more effective against the early wave of infection, which caused the wilt recorded September 11, than against later infection. This suggests that the late infection was due to inoculum that drifted in from neighboring plots. If such is the case, the soil treatments would probably be more effective on large plots or if supplemented by a spray program. The favorable effect of the bordeaux spray program, especially the heavier applications, appeared quite striking in the field. The contrast between plots 10 and 12 and their adjacent check plots was noticeable for a distance of several rods.

TABLE 1.—*Effect of various control measures on the incidence of wilt in test plots under severe epidemic conditions*

Control measures	Plot no.	Total germination	Seedling loss recorded on—							Total loss	Average total loss for a, b, and c
			Aug. 29	Aug. 30	Sept. 2	Sept. 11	Sept. 20	Sept. 25	Oct. 8		
		No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Seed dusts:											
Check	1a	592	0.8	3.4	5.2	87.2	3.4	0	0	100	
	1b	616	1.3	1.0	2.0	95.7	0	0	0	100	100
	1c	540	.5	1.6	4.5	89.6	3.8	0	0	100	
Zinc oxide	2a	464	1.3	2.5	2.7	89.8	3.7	0	0	100	
	2b	648	.5	.3	0	99.2	0	0	0	100	97.4
	2c	508	1.2	2.0	2.9	74.2	11.8	0	0	92.1	
Copper carbonate	3a	576	.8	3.6	2.4	83.3	7.5	0	0	97.6	
	3b	768	.5	.5	.5	92.0	5.2	0	1.3	100	89.9
	3c	540	.7	3.3	0	3.7	64.4	0	0	72.1	
Red copper oxide	4a	308	1.3	2.4	5.4	67.6	20.2	0	0	96.9	
	4b	440	1.3	0	4.1	8.0	82.0	0	1.1	96.5	96.0
	4c	544	0	2.1	1.1	23.6	67.9	0	0	94.7	
Soil treatments:											
Check	5a	532	.7	2.8	2.8	77.8	14.1	0	0	98.2	
	5b	528	.7	0	3.2	20.8	73.2	0	0	97.9	97.6
	5c	596	.5	14.9	2.1	24.7	54.6	0	0	96.8	
Bordeaux dust	6a	508	1.0	4.1	3.9	17.3	63.7	0	0	90.0	
	6b	588	.8	4.1	1.2	0	59.9	0	0	66.0	64.7
	6c	412	1.7	1.4	1.0	0	24.4	0	9.7	38.2	
Aluminum sulphate	7a	648	.3	1.1	2.0	0	3.1	9.2	3.1	18.8	
	7b	528	.9	1.9	2.3	0	66.4	0	0	71.5	35.2
	7c	516	1.1	1.1	1.5	0	3.9	0	7.8	15.4	
Check	8a	532	.4	4.7	2.8	79.0	11.3	0	0	98.2	
	8b	532	1.1	0	5.1	63.4	28.6	0	0	98.2	97.9
	8c	508	1.2	1.2	.6	82.4	11.8	0	0	97.2	
Sprays:											
Check	9	456	1.8	8.8	4.4	80.6	4.4	0	0	100	.....
Light bordeaux	10	468	1.1	3.6	1.5	2.1	60.5	0	0	68.8	.....
Check	11	492	2.8	2.4	90.5	0	0	0	2.3	98.0	.....
Heavy bordeaux	12	504	.4	0	0	0	0	10.3	0	10.7	.....
Seed dust:											
Check	13	254	0	2.8	3.6	93.6	0	0	0	100	.....
Copper carbonate	14	262	0	1.7	7.6	71.9	7.6	0	0	88.8	.....

It is apparent that the disease was controlled sufficiently for practical purposes either by acidification of the soil with aluminum sulphate or by the heavy application of bordeaux spray. However, these measures would seem to be economically feasible only in exceptional cases, e. g., in beds that are resown following a severe epidemic.

The acidification experiments suggest that severe epidemics of the disease rarely occur on acid soil. Observations made in forest nurseries in the summer of 1935 substantiate this conclusion. In the writers' opinion the disease can be avoided in most cases simply by selecting well-drained sites for the beds, with soil having an acidity of approximately pH 5.

SUMMARY

In the summers of 1933 and 1934 large patches of black locust seedlings (*Robinia pseudoacacia* L.) in a Virginia nursery wilted from a disease that had not previously been reported. In 1934 a few similar cases also were observed in a North Carolina nursery. In 1935 the disease was found in another North Carolina nursery and in an Alabama nursery.

The severest damage and the most characteristic symptoms of the disease develop in seedlings 1 to 3 weeks old. When the seedlings are a month old or more, new growth may suddenly wilt, but the plants as a whole usually recover.

Strains of *Phytophthora*, identified as *P. parasitica* Dast., were repeatedly isolated from wilted seedlings, and the disease was induced among healthy seedlings by spraying them with swarm spores and by infesting the soil with culture material.

Culture studies and inoculation experiments show that the liberation of swarm spores, encystment, germination, and penetration all take place in 4 hours, indicating that overnight rains provide conditions suitable for dissemination of the pathogen.

The disease was controlled in the greenhouse and in field plots by acidifying the soil to a pH value of 4.6 or by means of a spray program with bordeaux mixture. It is suggested that well-drained sites having a soil acidity of approximately pH 5 be selected for sowing black locust.

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